TRANSACTIONS

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TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

VOL 43 No 1 JULY, 1949

LABORATORY MEETING

of the Society held at the
Royal Army Medical College, Millbank, London,
on
Thursday, 17th March, 1949, at 7 30 p m

THE PRESIDENT,
SIR PHILIP MANSON-BAHR, C M G, D S O, F R C P,
in the Chair

DEMONSTRATIONS

ROYAL ARMY MEDICAL COLLEGE

Colonel A Sachs and Lieut -Colonel A N T Meneces

Heat hyperpyrexia in the United Kingdom

Three fatal cases of heat hyperpyrexia occurred in England in July, 1948, during a period of abnormally hot weather The macroscopic postmortem findings were identical with those seen in postmortems carried out in Irak and India Sections of different organs were shown to illustrate the histological changes found in the three fatal cases which occurred in England

Lungs —Congestion, oedema, haemorrhage and emphysema
Cardiac Muscle —Swelling, early hyaline change, and commencing fracture of muscle fibres
Kidney —Inter-tubular oedema
Suprarenals —Congestion and haemorrhage
Stomach —Mucosal congestion
Cerebrum —Round cell perivascular infiltration, haemorrhages and neuronophagia
Cerebellum —Thrombosis, disintegration and loss of Purkinje cells Purkinje cells also swollen and surrounded by oligodendroglia

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

DEPARTMENT OF PARASITOLOGY

Professor H. E. Shortt

Dientamoeba fragilis, free form isolated from a case of diarrhoea due to _Salmonella_ infection

Binucleate and uninucleate forms are shown with the characteristic arrangement of the nuclear chromatin as a ring of 4 to 6 granules with, sometimes visible, a small centrally placed granule which may represent the karyosome.

Professor J. J. C. Buckley

(a) **Cysticerci in liver and lung of ring-tailed lemur**

(b) **Living miracidia and embryonated egg of _Paragonimus_ from Indian leopard cat**

( ) **_Enterobius vermicularis_ in intestinal wall.** (Specimen sent by Dr A. Kertesz, City Hospital, Kano, N. Nigeria.)

( ) This massive infestation with cysticerci in the liver and lung was the cause of death of the lemur. The cysts are immature, only about 1 in 10 being found to contain a scolex, and this was incompletely developed. They are the larval stages of a species of *Taenia* probably of canine origin. The specimen was provided by the Zoological Society of London.

(b) Eggs of *Paragonimus* were found in the faeces of an Indian leopard cat at the Zoological Gardens, London, and after concentration were cultured in water for 21 days.

( ) This specimen is a section through the transverse colon of a 56-year-old Hausa man, who died of gangrene of the lung. A few chronic hypertrophic ulcers were found in the colon, and one of these has apparently been invaded by large numbers of adult *Enterobius vermicularis*.

Dr P. L. le Roux

Abnormal adaptations and development of schistosomes in experimental animals

I. Several mounted and unmounted specimens of *Schistosoma mansoni* and *S. mattheei* from mice and guinea-pigs were exhibited to demonstrate:—

(1) Hermaphroditism in *S. mansoni* and *S. mattheei* from guinea-pigs.

(2) Underdevelopment of females, especially the ovaries, in *S. mansoni* from mice, guinea-pigs and *Cercopithecus aethiops*, and in *S. mattheei* from mice and guinea-pigs.

(3) Underdeveloped males, aged 4 months, of *S. mansoni* from the lungs and heart in a mouse which harboured only males.

(4) From one to four sexually underdeveloped females of *S. mansoni* in the gynaecophoric canal of a single male *S. mattheei*. These were obtained when an attempt was made to interbreed the two species.

(5) Specimens from a case of intra-uterine infection which resulted when a pregnant mouse was injected intraperitoneally with cercariae of *S. mattheei*.

(6) Ovary situated just posterior to the middle of the body in specimens of *S. haematobium* from the bladder wall in an experimentally infected *Cercopithecus mitis kolbi*. The *in utero* eggs were more spherical than usual.

II. Macroscopic specimens showing the invasion of the urinary bladder of *Cercopithecus mitis kolbi* and *C. aethiops* by *S. haematobium* and *S. mansoni* respectively. *S. mattheei* has also been recovered from the same habitat in the golden hamster and the baboon here

III Microphotographs of the liver showing worm emboli caused by a too rapid destruction of the parasites in sheep that were heavily infested with *S mattheei* Rapid treatment may prove drastic, even fatal, in heavy intestinal infestations in Egypt and China

IV Males and females of *S mattheei* showing a marked decrease in size in a sheep which had been treated with Ant pot tartrate by the old method and interference with the portal circulation reduced

V Mounted tissues showing the eggs of *S mansoni* and *S mattheei* in the liver, lungs and the small intestine of a mouse which was exposed to *S mattheei* invasion after it had been infected with *S mansoni* for 7 months This suggests that this human species did not protect the mouse against the animal species Further experimentation revealed that *S mattheei* infections of short or long duration did not protect mice or guineapigs against *S mansoni*

VI A photograph of Bilharz's illustrations of "*Distomum haematobium*" was exhibited showing that he illustrated the common vector of endemic haematuria and not *S mansoni* The position of the ovary in the female illustrated by BILHARZ should perhaps be attributed to the fact that the parasite was immature or abnormal It was recovered from the portal vein

Photographs of eggs of "Bilharzia Capensis" by HARLEY (1864) prove that the spindle-shaped egg (Fig 12) is not an abnormal product of a disease stricken *S haematobium*

VII The influence of air and soil temperatures on the geographical distribution, and the seasonal discharge of cercariae, of human and animal schistosomes was illustrated by means of maps and temperature charts of mean air and soil temperatures at certain centres in known endemic and non-endemic areas The autochtonous cases of schistosomiasis haematobia, reported from India within the past, were due probably to the invasion of man by one of the animal species (*S suis*, *S indicum* or *S bomfordi*) which have eggs resembling those of *S haematobium*

VIII Maps, illustrating the convergence of human schistosomiasis and the tick-borne cattle disease heartwater, were exhibited

IX Specimens of wild and laboratory reared fresh water molluscs, accepted intermediaries of human schistosomiasis in Africa, were exhibited to illustrate various abnormalities which have appeared in locally laboratory reared specimens of *Physopsis africana*, *Bulinus truncatus*, *B tropicus* and *Lymnaea natalensis* There seems to be no valid reason for the recognition of more than one species of Physopsis in Africa The variations, in shape and size in laboratory reared *P africana*, prove that it is very closely related to *B truncatus*

Mr S S Qadri (introduced by Professor H E Shortt)

Myxosporidian parasite of an Indian fresh-water fish

1 *Myxosporidia* in kidney blood of *Clarias batrachus*
2 Myxosporidian cyst in kidney ,, ,,
3 Myxosporidian cyst in liver ,, ,,

Trypanosome of an Indian fresh-water fish

4 *Trypanosoma* sp in heart blood of *Clarias batrachus*
5 *Trypanosoma* sp in blood film ,, ,,

DEPARTMENT OF ENTOMOLOGY

Dr D S Bertram

Infection of the immature stages of the mite *Liponyssus bacoti* with *Litomosoides carinii*, the filarial parasite of the cotton rat

The protonymph of *Liponyssus bacoti* is susceptible to infection with *Litomosoides carinii*, but the intensity of infection is less than that obtained in

adult female mites. The intensities of infection for infective forms in a batch of protonymphs and in a batch of adult females taking up microfilariae from the same rat over the same time interval were as follows

Infected as	Per cent. infection rate	Mean number of worms per mite.	Maximum number of worms per mite
Adult females	78	5·96	64
Protonymphs	12	0·12	1

The difference in intensity is probably due to the smaller amount of blood ingested by the protonymphs.

Mr C. Garrett-Jones

The distribution of Auchmeromyia luteola in Africa

A demonstration of the life history of the fly *Auchmeromyia luteola* F was given at the Society's meeting year ago. This year map was shown of the recorded distribution of the species. This, like the laboratory experiments on the physiology of the maggot, indicates its tolerance of wide range of temperatures and humidities. The geographical distribution extends through central Africa from Air (lat. 18° N.) and El Obeid to Ngami and Durban (30° S). Some of the monthly isotherms in its localities range from 55 to 85 F., and their annual rainfall from nil to well over 80 inches. It has been found up to altitudes of 8 000 feet and there is one extra-continental record, all stages of the fly being found in the Cape Verde Islands.

Although this species, the only dipterous parasite specific to man, is of some medical interest, persistent enquiries have failed so far to elicit records from more than about half of its presumed range. This is due partly to national frontiers but partly to the fly passing unnoticed by medical and scientific workers.

Mr W H Potts (East African Tsetse Research Organization Shinyanga)

The distribution of the tsetse species in East Africa.

Mr POTTS exhibited a map now in course of preparation (scale 1 3,000,000) showing the distribution of the tsetse species in Eastern Africa. This map is one of three now being made, covering the whole of Africa, as the result of recommendations passed at an International Conference on Tsetse and Trypanosomiasis held at Brazzaville in February 1948.

The species of tsetse now known to occur in Eastern Africa are eleven *Glossina morsitans* Westw *G pallidipes* Aust., *G palpalis* Rob. Desv., *G swynnertoni* Aust., *G longipennis* Corti, *G brevipalpis* Newst., *G austeni* Newst., *G fuscipleuris* Aust., *G nigrofusca* Newst., *G fusca* Walk., *G tachinoides* Westw

The map showed *G morsitans* as the most prevalent tsetse of Eastern Africa, with *G pallidipes* occurring sporadically throughout its range and even further south (Zululand).

It also showed the linear character of the distribution of *G palpalis* in arid Eastern Africa, where it is confined to the neighbourhood of the infrequent permanent surface waters of this region—in marked contrast to its widespread occurrence in the wetter central and western portions of Africa

Other points illustrated by the map were the replacement of *G morsitans* in the very dry country of the north-east by *G longipennis* and *G pallidipes*, and the way in which isolated pockets of fly replace the broad belts in the northern, arid areas It is also worthy of note, how even such forest species as *G brevipalpis* and *G austeni* occur in such relict patches of forest as remain on the rivers and in other suitable places in some of these pockets Such occurrences suggest a former more widespread distribution of these species and their forest haunts, the moister conditions of the Pluvial periods, which in East Africa appear to have occurred at the same time as the Glacial epochs of Europe, would have rendered this possible

Dr R C Muirhead-Thomson

An experimental hut for testing residual insecticides against mosquitoes in the field

The demonstration showed the type of window trap cage designed, and photographs of experimental huts with window cages in position

When studying the effects of treating native houses with residual insecticides, it is essential to trap, in as natural a way as possible, the mosquitoes which escape from the house

This hut and window trap is designed on the principle that mosquitoes trying to leave the shelter of the house at any time between dusk and dawn are strongly attracted to faint light coming in from outside While hungry mosquitoes can enter the hut through innumerable minute crevices where the thatch roof rests on the wall, the only light coming into the hut is through a 1 foot square window opening over which the detachable trap is fixed

In houses treated with DDT in kerosene, or DDT dispersible powder, the window trap reveals that large numbers of *Anopheles gambiae* can feed on the occupants of treated huts, and escape unharmed

A correspondingly heavy dose of gammexane dispersible powder, P 530, showed a high kill of *A gambiae* inside the hut, with no indication of mosquitoes escaping unharmed, for at least 3 months after treatment

The present design of hut as used in both West and East Africa has mud walls and thatch roof built on a bamboo framework But this design could be modified to suit different types of housing in other countries

LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Department of Tropical Medicine

Professor B G Maegraith and Dr W H H Andrews

Paludrine and the treatment of falciparum malaria in England

Recent reports of relapses of falciparum malaria after paludrine therapy have led to the suggestion that paludrine should be reinforced with mepacrine

We have been using paludrine without reinforcement as a routine in uncomplicated attacks of falciparum malaria, in a dosage of mg 300 or 500 b.d. for 10 to 14 days. The clinical response in all cases has been good. Toxic effects noted on this dosage have been negligible, but nausea was present in a few cases.

Most of our patients were merchant seamen, and it has been possible to follow up only 30 cases treated with paludrine alone. There have been no relapses. Fifteen of these cases gave no history of previou malaria. (Table)

TABLE

LENGTH OF TIME OF FOLLOW-UP OF 30 UNCOMPLICATED FALCIPARUM CASES TREATED WITH PALUDRINE.

Months from cessation of treatment	6–12	12 4	24–36	36–47
Cases followed up ..	8	5	9	8

These figures are small but they surely indicate that over fairly wide range of strains, falciparum infections respond well to adequate paludrine treatment, and that the likelihood of relapse in random sample of trains n small.

Region in which the infection was acquired: West Africa, 23 Mediterranean littoral 2 India, 1; Doubtful, 4

Our method of follow-up was also shown. On leaving hospital each patient is issued with follow-up cards, three glass slides, and instructions on how to make blood films. They are also requested to make films before starting antimalarials. If the cards are not returned letter and questionnaire are sent later

Dr W E. Kershaw Mr W Crewe and Professor R M Gordon

The local reaction of the animal host to the bites of snakes and the stings of venomous creatures.

The literature concerning the composition and nature of the enom of bees, wasps, scorpions and snakes is extensive, and there are numerous accounts of the local and general signs and symptoms following the introduction of the poison. On the other hand observations on the histology of the local lesions produced by the natural stings or bites of these creatures are remarkably few

Sections were shown which had been taken from laboratory animals either 1 or 24 hours after the bite or sting had been inflicted.

The specimens showing the bites of *Glossina* and *Chrysops* were shown at previous meeting of the Royal Society of Tropical Medicine but were shown again for the purpose of comparing the lesions caused by the bites of blood-sucking insects with those produced by the sting of the wasp and scorpion, and the bite of the colubrine and viperine snakes.

A section through the skin and underlying muscle of guinea pig taken one hour after the sting of wasp (*Vespula vulgaris*) showed scanty cellular infiltration in the dermis,

the majority of the cells being eosinophils, with no oedema nor changes in the muscle A section taken 24 hours later showed much polymorphonuclear leucocytic infiltration, with but few eosinophils, a little adjacent necrosis in the muscle, and much oedema throughout the tissues

A similar section taken from a guineapig one hour after the sting of the scorpion (*Isometrus* spp) showed little cellular infiltration of the dermis, but marked coagulative necrosis of the muscles There was no haemorrhage evident The animal showed no obvious general symptoms at the time

A section from the white rat taken one hour after the bite of the green mamba (*Dendraspis viridans*) showed a large localized haemorrhage in the subcutaneous tissues and under the platysma, with some localized diapedesis nearby There was no evidence of the action of a haemolysin within an hour of the bite Near the haemorrhage and spreading along the interfascial planes there was a widespread coagulative necrosis, but no oedema and no inflammatory reaction The animal died about an hour after the bite

A similar section taken one hour after the bite of the gaboon viper (*Bitis gabonica*) showed a widespread diffuse haemorrhage (probably due to diapedesis) and oedema in the subcutaneous alveolar tissue and in foci in the muscles and in the deeper connective tissues There was much separation of the red blood cells and the margins of the extravasation were very diffuse, unlike the sharp demarcation of the haemorrhage following the bite of the green mamba The red cells were intact, and there was no coagulation In contrast with the bite of the green mamba, there was little necrosis of the muscles The animal died about one hour after the bite

Dr C A Hoare

The food habits of *Entamoeba histolytica*

Most clinicians still regard *Entamoeba histolytica* as an obligatory tissue-parasite, which invades the gut wall, with the production of gross or minute lesions, and feeds on erythrocytes and tissue elements However, there is a steadily increasing number of workers who believe that *E histolytica* can also live as a commensal in the lumen of the gut, without causing damage to its wall and feeding on micro-organisms and other faecal contents Their views are supported by observations on the behaviour of *E histolytica* under various conditions of existence Thus in amoebic dysentery the amoeba feeds on erythrocytes, while in cases of chronic amoebiasis and in symptomless carriers it ingests various micro-organisms and faecal debris In experimental infections of rats it shows every gradation from a commensal life, when it subsists on bacteria and cell-debris, to true parasitism, when it feeds on red blood corpuscles In monkeys this amoeba usually produces a symptomless infection and feeds on micro-organisms, while in cultures it may ingest starch granules as well In addition to phagotrophic nutrition *E histolytica* takes up food saprozoically, by absorption of fluid through the surface of the body

The host-parasite relationship in human amoebiasis has already been briefly discussed elsewhere (Hoare, 1947) *Trans R Soc trop Med Hyg*, **41**, 87), and it is proposed to deal with the commensal habits of *E histolytica* in a separate paper

The demonstration comprised a series of preparations illustrating the omnivorous habits of *E histolytica* under various conditions and in different hosts

Dr R C Rendtorff, Mr W R Jones and Dr G Robert Coatney (From the Laboratory of Tropical Diseases, National Institutes of Health, Bethesda, Md U S A)

Studies on the life-cycle of *Haemoproteus columbae*

The course of an infection of *Haemoproteus columbae* in pigeons was studied

Infections were produced ( ) by injection of suspension of macerated lung from naturally infected pigeon (*b*) by *injection of infected salivary glands taken from an infected pigeon fly* (*Pseudolynchia canariensis*) (*c*) by the bite of an infected fly

Two distinct patterns of infection resulted. When an adequate number of sporozoites were injected by method (*b*) or (*c*) gametocytes appeared in the peripheral blood 17–33 days later and multiplied rapidly to reach a peak of 10–38 per cent. *parasitization of erythrocytes*. When macerated lung was injected, gametocytes appeared 29–50 days afterwards, but only reached a peak of about 2 per cent. parasitization of erythrocytes. Other pigeons showed this latter type of infection after the injection of small numbers of sporozoites, or in the case of one pigeon after single bite by an infected fly

To determine the phase of an infection in pigeons optimal for the transmission of the infection to pigeon flies, the flies were allowed to bite an experimentally infected pigeon during the initial phase of its infection. The flies were allowed to bite the donor-bird for 2 days, then transferred to clean birds where they stayed for further 14 days.

The table gives a summary of the results of this experiment :—

TRANSMISSION OF INFECTION BY PIGEON-FLIES WHEN FED AT DIFFERENT PHASES OF A DONOR-BIRD'S INFECTION

Day of donor-bird's patent infection when flies were allowed to feed.	Development of sporozoites.	Pre-patent period* in recipient bird.	Type of infection developing †
3– 5	None detected	—	None
8–10		34	Low grade
10–12		38	
15–17		—	None
25–29	Present in 3/15	17	High grade
30–32	4/28	25	

Pre-patent period counted from time flies were removed from the recipient bird.
† Low grade less than 2 per cent. of erythrocytes infected. High grade more than 10 per cent. of erythrocytes infected

Charts were shown illustrating this experiment, and the two types of infection referred to above. A small brass fly cage used in the experiments was also demonstrated, and slides showing gametocytes in the peripheral blood, and schizonts in the lung and spleen were shown.

Dr A. Macpherson and Dr G M Findlay

Treatment of Vincent's infection with penicillin

A series of photographs and illustrations was shown to demonstrate the effect of penicillin on the various types of Vincent infection, cancrum oris, tropical ulcer gingivitis and infection of the skin resulting from human bites

Air Vice-Marshal T C. Morton

Kala-azar relapse following splenectomy

This patient contracted kala azar in Calcutta in December 1945 He received numerous courses of urea stibamine pentostam, pentamidine neostibosan and carbostibamide

(4) *Tunica erythroidea* (cremaster fibres stretching on tunica fibrosa). (5) *Tunica fibrosa* and (6) *Tunica vaginalis* are free from process. Two of the slides show granuloma infiltration in the cellulo-connective layer and lengthwise and crosswise sections of hypertrophied muscle fibres.

Professor E. J. King

Determination of haemoglobin and other blood constituents with the M.R.C. Grey Wedge photometer

TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE
Vol 43 No 1 July, 1949

ORDINARY MEETING

of the Society held at

Manson House, 26, Portland Place, London, W ,

on

Thursday, 19th May, 1949, at 7 30 p m

THE PRESIDENT,

SIR PHILIP MANSON-BAHR, C M G , D S O , M D , F R C P ,

in the Chair

PAPER

TROPICAL DISEASES IN BRAZIL

BY

PROFESSOR B MALAMOS, M D , D T M ,
Athens (Greece)

It is a great honour to have been invited by the President and the Council of the Royal Society of Tropical Medicine and Hygiene to read a paper on my impressions of my visit to Brazil The opportunity to visit Brazil was given me after the unanimous decision of the first General Conference of U N E S C O in Paris, December, 1946, to set up

(1) an international Scientific Commission in consultation with the Amazonian countries, Great Britain, Netherlands, France and the U S A , to investigate on the spot all aspects of the question of the establishment of an International Scientific Institute for the Hylean Amazon , and (2) to establish a Field Science Co-operation Office for Latin America

I am grateful to Dr JOSEPH NEEDHAM, F R S , Director at this time of the Natural Sciences Division of U N E S C O , for having chosen me to participate in these projects and for his advice and help during my work with U N E S C O

In May, 1947, Mr E J H CORNER, a British botanist who specialized for many years in tropical botany, and myself, were sent by U N E S C O for both purposes to Brazil We had the advice of different international consultants and leading scientists of the country Our headquarters were established in Rio de Janeiro During my 13 months' stay I was able to visit the interior of the States of São Paulo, Minas Geraes, and the Amazon Valley on two occasions

We were everywhere received with the greatest hospitality and had the co-operation and help of scientific and administrative organizations and of the local scientists. These visits to the interior provided an opportunity of becoming acquainted with the numerous epidemic and endemic diseases of these regions.

The establishment by U.N.E.S.C.O. of an International Scientific Institute for the Hylean Amazon Region was proposed by the Brazilian delegation. Hylean Amazon or Hylea refers to the area of tropical forest in the Amazon basin. This valley covers about four and a half million square miles and includes the great plain which stretches from the Andes to the Atlantic, part of the upper Orinoco, the three Guianas, Lower Tocantins, the sea coast of the State of Para and part of the State of Maranhão, the whole making up nearly one third of the total area of South America. This forest area is split up by the Amazon river and its tributaries. About half the area of the valley is Brazilian, the other parts belonging to Bolivia, Peru, Ecuador Colombia, Venezuela, and to British, Dutch and French Guiana.

The population in the Amazon valley is very sparse. The following figures for the Brazilian Amazonian States illustrate this —

State	Square miles.	Population.	Inhabitants per square mile
Para	471 000	1 150 000	2·44
Amazonas	595,000	515,000	0·86
Matto Grosso	447 000	510 450	1 14

Travelling by plane over this area one sees for hours only virgin forest, in most parts of which no human beings have yet entered. Very rightly ALBERTO RANGEL has called this region "inferno verde," the green hell. Characteristic of this tropical forest is the variety of species of trees in a given area, and the great size attained by many of them. LE COINTE emphasizes that in a hectare of wooded land it is usually easy to find 200 different species of trees.

The extent of the Amazon river and its many tributaries is amazing. Of some of these tributaries we have never heard the name. About 40,000 miles of these rivers are navigable on Brazilian Amazonian territory alone.

STRONG, SHATTUCK and WHEELER state rightly "that to summarize the virgin forest of the Amazon offers little hospitality to the traveller and one soon dies of hunger. During the dry season one suffers from thirst, when almost all the streams which traverse it are reduced to some few puddles of stagnant and brackish water. It is too thickly and too regularly grown to be grand or picturesque, and too silent to be cheerful. It breeds too much vermin to be agreeable and produces upon the traveller vague sensations of sadness, oppression and uneasiness, which cause him to breathe a sigh of relief or to

cry out with joy when chance conducts him to some camparina, or small prairie, or when he reaches the sunny bank of a stream with billows tumbling among the rocks of its yet imperfectly excavated bed "

The climate of the Amazon valley is very tiring and monotonous In this region, which extends for a few degrees of latitude to the north and south of the Equator, the four seasons of the year do not exist and the temperature is continually high throughout the year The temperature rarely exeeeds 34 to 35° C in the shade The annual average temperature was in Manaos, the capital of the State of Amazonas, for instance, 26 4° C in 1946 While there is little variation in temperature, the hot months in the lower Amazon last from September to January, the highest temperature occurring in October, April and May being slightly cooler

Although the temperature in the shade is not frequently very high, and not as high as is observed in many other parts of the tropical world, it is the constancy of the relatively high humidity which renders the climate especially debilitating and enervating The maximum humidity observed in Manaos was 99, the minimum 54 , and the average for 1946, 83 2 The nights are generally exhausting from the heat, which gives rise to restlessness and profuse perspiration

There are seasonal variations in the rainfall, causing a long wet and dry season The total rainfall in Manaos was in 1944 2,188 4 mm This leads to a general rise in the rivers from March to June The rise in the rivers in some places is enormous THOMAS points out that at Manaos the rise of the Rio Negro may amount to 50 to 60 feet At this time the valley may become a vast sea

Infectious and parasitic diseases are widespread in the relatively scant population of this large valley In the years 1939 to 1941, from the registered deaths in Manaos 46 16 per cent , and in Belem 43 74 per cent , were due to infectious and parasitic diseases I think that DJALMA BATISTA writes very rightly " that the visitor of the Amazon valley is fascinated by the greatness and magnitude of Amazonia, and especially by the daily struggle of its human population The life of this population—with few exceptions—is an anonymous Odyssey "

U N E S C O decided to take the necessary steps to establish an international scientific institute for the Hylean Amazon basin because this region is practically undeveloped Very few investigations have been made regarding the many surface and underground treasures of Amazonia It is stated that through development, exploitation, and health measures—despite the climate —100,000,000 persons could be settled in the Amazon valley In three international conferences in Belem (Para), Iquitos (Peru) and Manaos (Amazonas), the establishment of the Institute was decided and Manaos chosen as the site

The State of Minas Geraes, which I had the opportunity of visiting, is known for its many mines, its developed agriculture and its stock The state

is 229 000 square miles, with a population of 7,310 000 (1944) and a density of 32 inhabitants per square mile.

The State of São Paulo is the richest state of Brazil with most of the industry of the country and known for its coffee, cotton and rice plantations. São Paulo is 95,000 square miles, with a population of 7 733,500 (1944) and a density of 81·0 inhabitants per square mile.

This evening, I will try to give you, by showing coloured and black-and-white slides, a brief and comprehensive picture of the many public health problems which scourge this endless country. Brazil is 37 times larger than the United Kingdom, and has a population of about 46,000 000 only. The time at my disposal does not allow to go into details, and discuss bibliography and statistical material of all tropical diseases of the country. I will limit myself to the impressions of my stay in Brazil.

PUBLIC HEALTH ORGANIZATION

Brazil has the Federal administrative system, with a President of the Republic and a Federal Senate and Congress (House of Commons). Each of the 20 States has its Governor and State Parliament.

Public health is centralized under the Federal Minister for Education and Public Health. The Director of Public Health (Diretor de Departamento Nacional de Saude) was, during the period of my visit, the Professor of Tropical Medicine of the University of Bahia, Dr H. Fróes, and I wish to thank him for his co-operation and help. In a vast country with a relatively small population, more than 50 per cent. illiterate, and many hygiene problems, the combined centralization of education and public health in the same Ministry gives good results.

Very productive is the work of the National Institutes of Leprosy, Malaria, Plague, Tuberculosis and Yellow Fever. These National Institutes are Departments of the Ministry of Education and Public Health, and have their headquarters in Rio de Janeiro and their representatives in the different States. They are responsible for the campaigns and scientific observations involving the above-mentioned diseases. I had the opportunity to follow the many daily problems which they have to study and solve and how through systematic work, they begin to reap the fruits of their tireless activities.

The centre for biological research is the magnificent Instituto Oswaldo Cruz under Professor H. Aragão, in Rio de Janeiro. It is situated in the outskirts of the town in very extensive grounds. In the many laboratories various scientists work on many problems, especially tropical diseases. A small hospital facilitates their work.

Brazil has eleven medical schools. Some of them, especially the Medical Faculty and Faculty of Hygiene of the University of São Paulo, are located in very modern buildings with excellent laboratory facilities and good clinics.

In each State the Federal Government has his representative for public

health. Each State has its own public health service in charge of the different local problems of hygiene. In different States the budget of these services is rather limited; in others, as for instance São Paulo, they are able to develop widely and efficiently. The Directors of these State public health services are appointed by the State Governors and sometimes are not experts in public health.

We had the greatest assistance from the S.E.S.P. (Serviço Especial de Saude Publica). S.E.S.P. is an organisation set up in bilateral agreement between the Brazilian Government and the Institute of Interamerican Affairs in Washington. The object of S.E.S.P. is to develop public health and medical assistance for the welfare of the populations of the Amazon river and Rio Doce valleys. S.E.S.P. was established for 5 years in 1942, when numerous U.S.A. military personnel were stationed in the North of Brazil. The agreement has now been extended. The basis of this was that in the beginning the Institute of Interamerican Affairs was supplying the greater part of the annual budget. This quota diminished every year and the Brazilian increased. In 1948 Brazil provided five sixths of the annual budget. Bilateral agreements for similar purposes exist between the Institute of Interamerican Affairs and most Central and South American countries. S.E.S.P. has its headquarters in Rio de Janeiro, and has established different stations in the Amazon and Rio Doce valleys. They built public health, medical assistance centres, and hospitals in different parts of these two valleys. The rural districts of these regions have few or no resident doctors. I am grateful to the S.E.S.P. service not only for the help and co-operation of their staff, but also for the motor-boats put at my disposal to travel to different Amazon localities.

The work of the Rockefeller Foundation has been very successful in Brazil. They devoted themselves especially to the *Anopheles gambiae* and yellow fever campaigns. During my stay they were working only on the latter problem.

YELLOW FEVER

Yellow fever was till a few years ago one of the first—if not the chief—health problem of the country. *Aëdes aegypti* infected every year thousands of persons. Epidemics occurred often, generally with a high mortality. An epidemic of yellow fever appeared again in 1928 in Rio de Janeiro, causing many deaths. In the following years epidemics were recorded in different other towns. The Rockefeller Foundation, in collaboration with the National Institute for Yellow Fever, have achieved miracles in the last 20 years. Today, in many Brazilian towns, *Aëdes aegypti* does not exist, and from some States it has been eradicated. Trained staff regularly look for breeding places, and deal with these when found. In 1945, 52,464 areas of the country were controlled by these squads. Posts for viscerotomy have been established, and from every person dying of an acute febrile disease a small piece of liver is removed and sent to the Rockefeller Institute in Rio de Janeiro for pathological examination. In 1945, 1,279 viscerotomy posts existed.

The work of SAWYER and SOPER and their co-workers has shown by the mouse-protection test that yellow fever is spread over wider parts of Brazil than was originally known. The existence of jungle yellow fever was described in detail and it is generally recognized today that yellow fever—for Brazil at least—is an enzootic or epizootic disease of the forest animals. Especially monkeys (*Alouatta seniculus*, marmosets, cebus) seem to be infected. Different rodents have been found naturally infected, or the virus can be transmitted experimentally to them. In the forest the disease is transmitted by *Haemagogus* (especially *H. capricorni*) and *Aëdes leucocelaenus*, and possibly by some sabethine mosquitoes. Men going into or near the forest may be infected by these mosquitoes and can carry the virus to rural or urban localities. If *Aëdes aegypti* is present in these places an epidemic of yellow fever may follow and the cycle man–*Aëdes aegypti*–man is established.

In the last years very few cases of yellow fever have occurred yearly in man in Brazil, and these are diagnosed clinically or through viscerotomy. In 1945 116 were recorded and in the last years the number is less than 100. This decrease has not only been attained by the increased knowledge of the epidemiology, the antistegomyia campaign, the viscerotomy service, but also by the yearly increasing number of vaccinated persons. Nearly 5,000 000 people have been vaccinated in Brazil. The vaccine is 17D virus cultured in developing chicken embryos. The vaccine is prepared in the Rockefeller Institute in Rio de Janeiro. The vaccination is today absolutely harmless and not followed by a hepatitis. A certain number of vaccinated persons complain on the fifth to seventh—generally on the sixth—day of headache and a slight febrile reaction. In earlier years a delayed jaundice was observed among certain of the vaccinated. FOX, PENNA and PARA (1942) have given a detailed account of the icterus and hepatitis, following yellow fever vaccination in Brazil. In the 1939 outbreak, 27 per cent. of 304 persons vaccinated became icteric, for the most part during the fourth or fifth month following vaccination. In 1940 there were 1 072 cases and 24 fatalities. After the introduction of another strain of virus no jaundice or hepatitis is known to have occurred in Brazil.

In about 50 per cent. of persons vaccinated with the 17D strain, traces of it were found in the circulating blood after vaccination. It was proved, however, by WHITMAN that this concentration is too low to infect mosquitoes. The vaccination is effective for at least 4 years, probably longer.

TUBERCULOSIS

Tuberculosis is not a special tropical disease and should not be mentioned in this paper. As it is considered to be the greatest public health problem of the country, with about 100,000 fatal cases yearly—coming even before malaria and worm diseases—you will allow me to say a few words.

In most of the States the tuberculosis deathrate is higher than that from other infectious and parasitic diseases. The poor hygienic conditions in many

FIG 1—Hut infested with infected *Triatoma* All children living in this hut and the dog are infected with *Tr cruzi* Bambui Lavapes (Minas Geraes)

FIG 2—South American Leishmaniasis Destruction of the nose Ulceration of the upper lip Case seen at the University Clinic for Tropical Diseases Belo Horizonte (Minas Geraes)

FIG 3—South American Blastomycosis Splendore de Almeida Disease Destruction of upper and lower lips Case seen in University Clinic for Tropical Diseases Belo Horizonte (Minas Geraes)

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FIG. 4.—Pinta. Second stage. Case seen in Santa Casa Misericordia Hospital, Manaos (Amazonas).

FIG. 5.—Pinta. Third stage. Depigmentation of forehead. Case seen in Santa Casa Misericordia Hospital, Manaos (Amazonas).

FIG. 6.—South American Leishmaniasis. Multiple sores on face, upper and lower limbs. Was mistaken for leprosy and for fortnight isolated in leper colony. Case seen in Sesp—Hospital Belem (Pará).

rural districts and the slums (favellas) of the towns—even of Rio de Janeiro—where overcrowding, mostly among coloured people, and inadequate diet, favour the spread of the disease

Beds for cure and isolation of the infected are rather insufficient The Department of Health of the Ministry of Education and Public Health have launched, together with the National Institute of Tuberculosis, a campaign against the spread of the disease The Federal Government is willing to supply the whole or greatest part of the cost of establishing sanatoria if the State Governments are willing to cover the maintenance costs Repeatedly I was told that a great part of the population have a feeling of fear and shame regarding tuberculosis, and hide the infection

MALARIA

Malaria is, of all tropical diseases, the main problem No State is free from malaria, and it is even found in the outskirts of Rio de Janeiro Benign tertian is prevalent , then comes subtertian There are few cases of quartan It is amazing how extremely rarely blackwater fever is found Many malariologists have never seen a case Even years ago, when there was no other treatment than quinine, blackwater fever was a rare exception

In the Amazon valley malaria is prevalent The following numbers will give an idea about the extent

BELEM, capital of the State Para

Deaths from—	1942	1943	1944	1945	1946	1947
Malaria	504	452	458	497	368	317
Tuberculosis	640	788	876	685	705	688

MANAOS, capital of the State Amazonas

Deaths from—	1940	1941	1942	1943	1944	1945	1946	1947
Malaria	359	375	296	309	470	383	341	288
Tuberculosis	258	279	271	292	293	291	314	260

MANAOS Municipality (Population 1940, 109,022, 1947, 120,711)

Deaths per 100,000 inhabitants—	1940	1941	1942	1943	1944	1945	1946	1947
Malaria	329 3	338 1	263 4	270 9	406 1	326 2	286 3	238
Tuberculosis	238 5	252 0	250 1	256 9	253 2	247 8	263 6	215

In 1946 12 5 per cent of the examined population of Manaos had a positive blood or enlarged spleen

The blood and spleen indices of different districts of the town Manaos for 1946 were the following —

Adrianopolis	31 7 per cent	Flores	35 7 per cent
Bilhares	74 1 ,,	Matinha	48 1 ,,
Cachoeirmi	22 2 ,,	Sao Raimundo	55 0 ,,

The principal vectors of malaria in Brazil are *A darlingi*, *A tarsimaculatus* (*aquaesalis*) and *A albitarsis* There are some other species of minor importance

A darlingi is the vector in the interior It is responsible for the greatest percentage of infections of the country L M DEANE, O R CAUSEY and M P

DEANE point out that its larvae breed by preference in large and deep water collections, situated on the fringe of forests. Although *A. darlingi* larvae are found to be more widespread during the rainy season, when they can breed in small and shallow water collections, well exposed to the sun, they disappear from such breeding places during the dry season, to maintain themselves only in permanent foci such as reservoirs, broad, deep and slowly moving streams, lakes and large ponds bordering forests and bushy areas. In these permanent foci the larvae are to be found chiefly in the sunlit or partially shaded sections, being rare in the densely shaded portions. They breed not only along the margins of water collections, but also on the surface of deeper parts, away from the margins.

Such peculiarities in the biology of *A. darlingi* explain why this mosquito is easily found in houses throughout the year in localities near which there are permanent water collections suitable for breeding. *A. darlingi* foci were generally found less than 500 metres from the closest houses, but longer distances up to 2 km. have been recorded. *A. darlingi* is the most domestic species and was found more numerous in houses during the night than the day.

A. tarsimaculatus (*aquasalis*) is distributed along the coast with preference for brackish water breeding places, because of the resistance of its larvae to highly concentrated solutions of sodium chloride. *A. tarsimaculatus* is found only within a narrow coastal band, in rivers up to the point where the influence of tides is felt, or in places where, although far inland, ground water collections are found with a high chloride content. The breeding places of *A. tarsimaculatus* are small, sunlit or partially shaded collections of water usually brackish, such as ponds formed by the overflow of brackish water streams during high tides, or rain pools, ditches, lagoons, borrowpits, car and animal tracks on salty ground. The water has generally a content of 0·2 to 1 per cent. of chlorides, sometimes even a concentration of 1·5 per cent. *A. tarsimaculatus* is less domestic than *A. darlingi*. It shows a definite preference for late hours of the night in houses.

Finally *A. albitarsis* must be mentioned. In the Amazon, *A. albitarsis* var *domesticus* is especially found. The larvae of *A. albitarsis* can breed in many types of water collections, but are chiefly found in marshes, in grassy spots along the margins of rivers and in lagoons rich in organic matter.

The control of malaria in Brazil is a very difficult problem. In the Federal District I was shown by the Instituto Nacional de Malaria the campaign measures, consisting mainly in drainage and mepacrin treatment (in malignant tertian combined with pamaquin) of the infection. Lately aralen (chloroquine) has been used. Every year DDT is employed in the country on a larger scale. DDT spraying has been carried out only from airplanes (helicopters) but, as I was told, the large extent of waterways and distribution of the breeding places prevent favourable results. In some southern districts only this method gave good results. In the Amazon valley spraying from the air would be a waste

of work and money The method of choice is the spraying of all the houses in an endemic area In the Amazon valley the S E S P service has made from 1945 different trials in smaller areas, and obtained very favourable results

S E S P undertook in 1947, in Belem, an experiment to spray only a corridor with DDT near the main breeding places of *A darlingi* in this town, and to prevent by this the spread over the whole town In Belem, malaria is transmitted both by *A darlingi* and *A tarsimaculatus* (*aquaesalis*) This experiment did not yield good results as *A darlingi* by passed the corridor and established itself in new breeding places In 1948 S E S P started an extended programme to spray the towns of Belem and Manaos It was intended to spray all the houses of the infected parts of both towns In Manaos 10,000 of about 16,000 houses were to be sprayed, in Belem, 20,000 For this mass spraying a DDT solution in triton and not in kerosene will be used as transport costs are reduced by about 50 per cent The solution was gramme 35 DDT, gramme 4 triton and 100 c c xylene, dissolved locally before application in water to give a 5 per cent solution gramme 2 15 DDT must be used for m² In the Amazon valley three sprayings per year are necessary

For malaria treatment, mepacrin is used generally Lately, arralen (chloroquine) has been tried on a large scale In the Amazon valley R M MEIN and P N S ROSADO have used the new synthetic drugs and arrived at the following conclusions —

(*a*) CAM-AQl (4(3'-diethylaminomethyl-4'hydroxyaniline)-7-choloquinoline dihydrate)
(*b*) CHLOROQUINE (SN 7618 or 7-chloro 4-(4 diethylamino-1'methylbutylamino quinolein)
(*c*) PALUDRINE Hydrochloride (M 4888), and
(*d*) OXYCHLOROQUINE (SN-8137-5)
are all useful for malaria treatment

CAM-AQl was superior to the others for the following reasons —

(1) Quicker disappearance of fever,
(2) Parasites disappeared sooner from the peripheral blood,
(3) Symptoms subsided earlier,
(4) Hospital period was reduced, and
(5) Only one CAM-AQl dose is necessary for sterilization of the blood (8 tablets gramme 0 05 = gramme 0 4 for adults at once)

Special mention must be made of the serious outbreak of malaria in the north-east of Brazil after the arrival of *A gambiae* from Africa in 1930 Serious outbreaks followed in 1930 and 1931 from Natal (Rio Grande do Norte) to the interior The first organized campaign resulted, apparently, in eradication From 1932 to 1937 it was more or less quiescent until it encountered more favourable conditions in the Assu and Apodi valleys of Rio Grande do Norte and the larger valley of the Jaguaribe in Ceará In 1938, terrible outbreaks of malaria, with a high fatality rate, occurred in these two States (Rio Grande do Norte, Ceará) The Government organized a special antimalaria service which, with the co-operation of the International Division of the Rockefeller Foundation, undertook to organize a campaign of species eradication against

A. gambiae With paris green and pyrethrum against the larval and adult forms, initially concentrated on the peripheral and frontier zones, *A. gambiae* was stopped, its invasion beaten back, and finally it was eradicated from the known infested area in less than 2 years.

Very interesting is a special form of malaria encountered in the south, the Bromelia malaria. This form is endemic in the States Santa Catarina Parana, and some districts of Rio Grande do Sul. *Bromelia malaria* is characterized by the fact that the *transmitting species do not breed in water collections* on the earth surface, but in the stagnant rain water in the leaves of bromelia. Bromelia are epiphytes of the trees of the extensive forests of these regions. *A. bellator A cruzii* and *A homunculus* are the species breeding in the leaves of bromelia. The campaign against this malaria form is very difficult as any spraying—even with DDT—is without success. The only efficacious measure is to destroy the bromelia. *Squads climb the trees and cut the* bromelia. They have counted up to 3 000 bromelia on one tree. The National Institute of Malaria is preparing a plan to destroy them by burning the forest in a perimetric zone of some hundred yards around the endemic foci, with following reforestation

LEISHMANIASIS.

Two leishmaniasis forms occur in Brazil. *Leishmaniasis brasiliensis* is much more common than kala-azar

Kala-azar —There are no kala-azar epidemics in the country as in the Far East. A few endemic cases are seen in different parts of the country mostly in children. The epidemiology resembles the epidemiology of the disease in the Mediterranean basin. Dogs have been found infected.

Leishmaniasis brasiliensis (Espundia). This is common in Brazil. The *incidence in the States São Paulo and Minas Geraes is higher than in the northern* States. In the Amazon basin few cases are seen whereas in the Peruvian part east of the Andes they are rather numerous.

Pessoa, who has for years studied the disease, is of the opinion that it is a colonization disease. He has shown in maps which he has published, that the infected regions of the State São Paul have today moved to the periphery with the increasing colonization and deforestation of this State. Regions in which *previously numerous cases* were occurring are practically free today from the disease. It seems that chiefly persons going into or living on the borders of the forest are infected. There must be an animal host of the disease but it has not been demonstrated. The principal vectors are *Phlebotomus migonei, P whitmani P pessoai* and, in the State of Rio de Janeiro, *P intermedius*

The disease resembles, in its *early stages, oriental sore. One or several* boils are found. They are situated in the face or the other uncovered parts of the body At this stage the detection of the parasite, *Leishmania brasiliensis* is easy When the disease advances, and the mucous membranes begin to be

affected, then the demonstration of *L brasiliensis* is more difficult In the forms with ulceration and destruction, especially of the nose, the lips, mouth and palate, it is sometimes impossible or extremely difficult to detect the parasite, even in stained sections Sometimes nodular forms of the disease are seen with nodules on the face, nose, ears, hands and legs, resembling leprosy In Belem I saw a case in a young man which was mistaken for leprosy, and sent for more than a fortnight to a leper hospital, through the detection of *L brasiliensis*, the diagnosis was finally made

For diagnosis, Montenegro's reaction, with an antigen of killed leptomonas in suspension, is used intradermally Only reactions persisting for at least 72 hours should be counted as positive CID FEREIRA LOPES and J F LEANDER found the reaction in 71 4 per cent of mucocutaneous cases positive In cutaneous cases it was positive in only 36 1 per cent In all control cases, Montenegro's reaction was negative

For treatment, antimony is used, but the results obtained are in dispute

CHAGAS DISEASE

Chagas disease—South American trypanosomiasis—is a great epidemiological problem in different States of the country After the first description of the disease in the State of Minas Geraes by CARLOS CHAGAS in 1909, it was generally thought for years that only few endemic cases exist, and that South American trypanosomiasis is a minor public health problem The investigations of numerous Brazilian scientists, especially EM DIAS, have shown that Chagas disease is widespread, and in some foci a great percentage of the population is affected No specific efficacious treatment exists at present, and yearly numerous persons, especially children and young adults, die of the acute stage or of the heart complications of the disease Chagas disease is prevalent in the States Minas Geraes, São Paulo, Rio Grande do Sul, Parana, Bahia, but cases have been described from practically all Brazilian states There are localities where even 50 per cent. of the population have been found infected In Bambui, for instance, a small town of the State Minas Geraes, with a population of about 3,000, nearly 500 persons have been found infected

In the acute stage of the disease fever generally occurs for few weeks At this stage *Trypanosoma cruzi* can be detected in the blood In a number of acute cases the initial lesion, called chagoma, is found The chagoma is usually situated on the face with oedema of the eyelids In other cases the chagoma may be found on the arms or legs As the infection generally takes place during childhood, the acute stage and the chagoma are seldom seen in adults A number of those infected do not survive the acute stage In the greater part the chronic or latent stage develops after the acute, and continues for years and decades As the multiplication forms (*Leishmania* forms) of *T cruzi*, are chiefly localized in the heart muscle, cardiac troubles develop in a number of patients Extra systoles, arrhythmias, different types of heart block

(especially right branch block), Adams Stokes syndrome, with acute heart syncopes, are common. Goitres are often seen in chagas patients, and it was originally thought that there is a thyroid form of the disease. Most investigators today are of the opinion that there is no relationship and that the goitre is the result of a low iodine content of the water of these regions.

Besides the direct detection of the parasite in the blood, the following methods are employed for diagnosis (1) Xenodiagnosis (2) a complement fixation reaction and (3) inoculation of blood into mice and guineapigs.

The vector of the disease is different species of the bug *Triatoma*. In some regions *Triatoma megista* in others *T infestans* prevail. In Bambui for instance from 58,867 captured *Triatoma*, 4 722 were *T megista* and 54 145 *T infestans*. The vectors are found generally in primitive types of huts (called cafúas), which are built from cane and earth. E. DIAS and his co-workers have shown that in Bambui most of the human cases occur in the periphery of the town, where the type of houses and huts is more primitive. Of 293 of these huts, 195 were found infested with *Triatoma* 133 (70-77 per cent.) contained *Triatoma* infected with *T cruzi*. In the centre of the town only in 87 (20 per cent.) of the dwellings were *Triatoma* present. The number which can be caught in a hut is amazing 200 to 300 is a common catch, and E. DIAS states that he captured even 3 000 The *Triatoma* are to a high percentage infested with *T cruzi*. Percentages of 30 and 40, and even higher have been recorded.

Extensive trials have been undertaken to combat the vectors with different insecticides. DDT gave no success, and it seems that gammexane is more efficacious. The bugs are killed by gammexane and their abdomen appears swollen.

Different animals are hosts of *T cruzi* Dogs, cats are infected, and the natural hosts seem to be the gamba, tatu (armadillo, dasypus) and opossum.

MYCOSES.

Numerous forms of mycosis are found in Brazil. Various scientists are investigating them, and OLYMPIO DE FONSECA's book is an excellent guide

South American blastomycosis or Splendoro de Almeida disease (LUTZ) is often encountered The parasite of this blastomycosis is *Paracoccidiodes brasiliensis*. The parasite spreads through the lymphatics. There i localized cutaneous form, with swollen lymph glands, and a generalized form. The latter may develop as a continuation of the former local form. L. BOGLIOLO emphasizes that the primary infection occurs *mostly in the mouth cavity from* the tonsils or an apical dental granuloma spreading to the lymphatics and lymph glands of the submandibular region or the neck, and developing on face or neck as ulcerative cutaneous lesions. Cutaneous lesions on the limbs or trunk are more seldom found. When the disease becomes generalized, the lungs and bones are often affected and usually within some weeks or months it proves fatal. Sulpha drugs in high and prolonged dosage have been successful, but a number of cases relapse.

Chromoblastomycosis is a localized mycosis, with a chronic but not fatal course Papillomatous conditions develop on legs or feet The disease is more prevalent in men than women engaged in agriculture The sole of the feet generally escapes The disease is common in the Amazon valley The parasite is called *Phialophora verrucosa* and *Acrotheca pedrosa* In another terminology, the names *Fonsecaea compacta* and *Fonsecaea pedrosa* are used'

Mossy Foot—Most investigators consider that mossy foot, or Amazon foot, first described by W THOMAS in the Amazon valley, is identical with chromoblastomycosis Others separate the diseases The legs and feet are covered in mossy foot with warty outgrowths resembling barnacles, which are vascular and sometimes painful Usually they are papillomatous but occasionally pedunculated

Sporotrichosis—Few cases of sporotrichosis are found in Brazil The parasite *Sporotrichum (Rhinocladium) beurmanni* produces gumma-like swellings, specially on the limbs and occasionally only on the trunk These swellings enlarge and ultimately break down, leaving ulcers Generalized cases of the disease are rare Iodine is useful for treatment

WORM DISEASES

The main hygiene problem, after malaria, is worm disease In many States very high indices of infestation, reaching sometimes nearly 100 per cent, may be found in some areas In Manaos, for instance, in 1946, of 758 school children examined, 301 (41 8 per cent) were found positive for *Necator americanus* Of 663 persons examined in the same town, 31 07 per cent were positive for *Ascaris lumbricoides*, 28 05 per cent for *Ancylostoma duodenale* and *N americanus*, 12 21 per cent for *Trichuris*, and 1 35 per cent for *Strongyloides stercoralis* It is amazing to see how many children, even of the better class, are barefoot in Manaos, look anaemic and have reduced body weight It is interesting to note from the other side, that health education and the application by the population of the suggested prophylactic measures—even in heavily infested areas—give good results, and the children look healthy, with normal weight and good colour The greatest percentage of worm infections is due to *A lumbricoides*, then come *N americanus* and *A duodenale*, and to a lesser degree *Trichuris* and *Strongyloides* Cysticercus cellulosae (*Taenia solium*) and *Echinococcus* are rare

Schistosomiasis mansoni—In some regions of the country *Schistosoma mansoni* is widespread, as in the States of Minas Geraes, Bahia, Pernambuco In others, as in São Paulo, Amazonas, Para, only few localized foci and cases are found In some of the heavily infested areas a great number of persons (up to 80 per cent) are found infected In Belo Horizonte, the capital of the State Minas Geraes, the infection rate was, in 1920, only 1 per cent and in 1943, 11 per cent

Australorbis globatus is, in Brazil, the vector of *S mansoni* It is interesting

that near Belo Horizente an artificial lake (Pampulha) was made a few years ago and hundreds of bathers were infected in this lake. All measures against *A. globatus* were unsuccessful. One day they all disappeared from this lake of themselves, and bathing is today safe.

Schistosomiasis is generally chronic, with gastro-intestinal disturbances and a high eosinophilia. Sometimes acute cases (mass infections) are noted which end fatally in a few weeks. Persons with a swollen abdomen are occasionally seen.

Filariasis bancrofti occurs in different parts of Brazil. Belem (Para) and Bahia are the towns in which the disease is most common. The investigations of O. R. Causey, M. P. Deane, O. da Costa and L. M. Deane have shown that of 5 000 persons examined from various sections of the city Pelem (Para) 541 (10·8 per cent.) harboured *microfilariae*. Elephantiasis was observed in 1 3 per cent. of the examined persons. Of those found infected with microfilariae only about 12 per cent. had clinical symptoms. The principal vector was found to be *Culex fatigans*. Among 1·014 dissections, 118 (11·6 per cent.) were positive for filaria larvae. *A. darlingi* and *A. tarsimaculatus* (*aquasalis*) were also found naturally infected. Experimental infection was produced in *C. fatigans*, *A. darlingi*, *A. tarsimaculatus* (*aquasalis*), *A. oswaldoi*, *A. triannulatus* and *A. albitarsis*.

Clinically elephantiasis of the legs, feet, scrotum, vulva and breasts, is seen. The cases I had the opportunity to examine were less advanced than the cases with massive involvement described from Africa and the Far East. Different mycoses and erysipelas often develop on the swollen legs and feet.

SMALL POX AND ALASTRIM

Despite the vaccination campaign, a number of the population has yet not been vaccinated. In most States endemic cases of smallpox occur. The mortality is rather low and I was told in Belo Horizonte (Minas Geraes) that it seldom exceeds 1 to 2 per cent. The disease is clinically absolutely characteristic. Penicillin and sulpha drugs seem to be of value as a prophylactic and therapeutic for the developing bacterial complications in the suppurative stage. Persons with smallpox scars are not unseldom encountered, especially in the interior of the country.

Alastrim.—It seems that a number of the cases registered as smallpox are really alastrim. The disease is endemic in South America and the West Indies. Smallpox and alastrim resemble each other clinically, alastrim being less severe in its general clinical manifestations.

TYPHUS

Typhus exanthematous neotropicus occurs in the States of Minas Geraes and São Paulo, but has been found also in some other States. It resembles in its epidemiology and clinical manifestations Rocky Mountain spotted fever

and has a high fatality rate up to 80 per cent The clinical manifestations are identical with Rocky Mountain spotted fever Men are infected in the fields, forests, in or around their habitations Different animals are hosts of the virus O MAGELHAES and EM DIAS state that domestic dogs (*Canis familiaris*), wild dogs (*Cardocyon thous*), forest cats (*Felis wiedi*), coati (*Nasua narica*), furao (*Orison vittatus*), tatu (*Dasypus novemcinctus*), goats, agouti (*Dasyprocta*), and the wild rabbit (*Sylvilagus brasiliensis*) are the animal reservoirs of the organism O MAGALHAES separates three strains of *Rickettsia brasiliensis* (1) the strain VB, the classical, fixed, Brazilian strain, and (2/3) weaker strains VA1 and VA2 The disease is transmitted by the ticks *Amblyoma cayennense* (feeding with preference on dogs), *A brasiliense*, *A striatum* and, as O MAGELHAES claims, also by *Cimex lectularius*

SPIROCHETOSES

Different forms of spirochetosis occur in Brazil Syphilis is not a true tropical disease, but a brief mention is made as it is widespread in the country All three cutaneous stages and the gummatous, cardiovascular and nervous system complications are frequent In Belem (Para), for instance, about 25 per cent of the persons examined in the S E S P hospital had a positive serological reaction, which in some of the cases may be due to other diseases (*i e*, malaria, pinta, yaws) than syphilis From other areas even higher percentages of syphilis morbidity are recorded Among the population there is no fear or shame regarding infection, and treatment is openly discussed A great campaign has been launched lately by the Ministry of Education and Public Health

Different foci of yaws and pinta are found in the country Ulcus tropicum is widespread, especially in the north and interior of the country Few cases of Weil's disease and sodoku are seen

Yaws —Foci of yaws (called locally boubas or catita) exist in different States In some of these foci, as in the north-east of the State Minas Geraes and the State Rio de Janeiro, numerous cases are noted In the Amazon valley the disease is rather rare It is found in rural zones and is restricted to the poorer classes Predisposed are areas with a hot and humid climate near the forest In open country, with a hot and dry climate, yaws is very rare In some of the foci the epidemic is a recent one, with no relation to cases in Indians In such foci the majority of cases occur in adults and not in children as is generally seen, because of the acquired immunity of adults In the focus in the north-east of Minas Geraes, CID FEREIRA LOPES found, of 651 examined, the disease in 10 2 per cent of children aged 0 to 5 years, in 31 4 per cent aged 5 to 15 years, and 58 2 in people aged more than 15 years

Hereditary transmission was not found The initial lesion, framboesoma, is not infrequently seen CID FEREIRA LOPES emphasizes that in his observations 88 7 per cent of the examined were in the second stage and did not consult a doctor during the first stage He is of the opinion that fever, headach

and other *general symptoms* are rare exceptions in the first stage. The primary lesion framboesoma, is in its morphology not identical with the eruptions of the secondary stage. The framboesoma is larger flatter situated in the majority of cases below the knees, *and often causing a notable lymph node* reaction. This lymph node enlargement, of the size of a pigeon egg or even larger is only a little painful. The framboesoma is often surrounded by a whitish areola, with dry rough, keratotic or furfuraceous skin. This *areola* is not seen around the secondary lesions. In various cases the initial lesion starts from a tropical ulcer. During the second stage, granulomas of various size from lenticular varioloform to 3 to 5 cm. in diameter prevail. Hyperceratosis, called "crab" of the palm and sole, paraceratosis with a circinate of serpiginous appearance and *infiltrations* around the nails, are common. Third stage manifestations are rare. Ostitis and periostitis (especially of the tibia) and gummatous forms are occasionally seen. Gangosa is very rare and gundu *and juxta articular nodes seem not to occur. The treatment of yaws is very* efficacious with penicillin, neosalvarsan and bismuth. The Instituto Oswaldo Cruz has undertaken experiments and obtained cures with 200 units of penicillin daily.

Pinta called in Colombia "carate" and in Mexico "mal del pinto," is endemic in some localities of the Amazon valley *especially* the State of Amazonas, and is called "puru-puru" by the population. In the Peruvian part of the Amazon the disease is more common than in the Brazilian. In Brazil, pinta has not the same extent *as in Colombia and Mexico, whereof the persons examined,* 4 per cent. and 11 per cent. respectively were found positive. The disease is caused by *Treponema carateum* or *T herrejoni*. Pinta seems to be transmitted *directly* by contact and no insect vector is required. Clinically an initial papule appears, reaching a diameter of 1 cm. in a month and then continuing to spread peripherally. Secondary lesions, the pintids," appear around the primary papule spreading to other parts of the body. Progressive hyperpigmentation is noted and later depigmentation giving rise to different colours or vitiliginous spots over the body. The spots *vary in size and shape being* round, oval or irregular. In the tertiary stage (dyschromic) achromic or pigmentary spots, erythema, keratoderma and atrophy are found. Treatment with *neosalvarsan bismuth and penicillin* gives good results, and the lesions subside quickly but the atrophic vitiliginous spots remain unaffected.

LEPROSY

Leprosy is widespread over the country. Nearly 50 000 cases are registered in Brazil, being about one case in 1,000 inhabitants. The following chart will give an idea of the distribution *in the different* States —

CASES REGISTERED DECEMBER 31ST 1944 IN BRAZIL.

Alagoas	47	Mato Grosso	593	Rio Grande do Norte	17
Amazonas	-,010	Minas Geraes	10 555	Sul	855

Bahia	183	Paraiba	144	Santa Catarina	566
Ceara	1,174	Parana	1,398	São Paulo	16,686
Espirito Santo	838	Para	3,701	Sergipe	69
Federal District	2,651	Pernambuco	488	Different Territories	513
Goias	1,653	Piaui	232		
Maranhao	1,255	Rio de Janeiro	1,139		

In 39 leper asylums 20,719 of these cases were isolated on December 31st, 1944

J B RISI calculates the percentage of contagious cases in different Brazilian states as follows —

Para	31 06	Rio de Janeiro	55 09	Federal District	67 60
Maranhao	41 37	São Paulo	61 04	Rio Grande do Sul	69 33
Espirito Santo	44 00	Santa Catarina	62 00	Minas Geraes	70 00
Ceara	55 09	Rio Grande do Norte	67 40	Sergipe	73 69
Bahia	75 00	Paraiba	75·00	Piaui	77 00
		Parana	80 00		
		Pernambuco	88 30		

It is known that the incidence of leprosy prevails in men and different numbers are published of the relationship in both sexes Brazilian statistics from various States and leper asylums vary between 1 1 and 2 5 for the relation male female

The following chart shows the incidence of the disease in the different age groups

AGE AT WHICH THE FIRST LEPROSY SYMPTOMS APPEARED

Ages	Brazilian No	Brazilian Per cent	Foreigners No	Foreigners Per cent
0 to 5	292	(1 7)	2	(0 4)
6 „ 10	1,141	(6 6)	13	(0 3)
11 „ 20	4,688	(27 3)	121	(2 8)
21 „ 30	5,266	(30 7)	504	(12 0)
31 „ 40	3,418	(19 9)	891	(21 3)
41 „ 50	1,478	(8 6)	1,104	(26 3)
51 „ 60	609	(3 5)	868	(20 7)
More than 60	235	(1 3)	680	(16 2)
Total	17,127	(100)	4,183	(100)

The difference between the Brazilians and foreigners in this chart, which shows that the percentage in earlier years of life is higher in Brazilian than in foreigners must not be attributed to a greater predisposition of the Brazilian, but to greater possibilities of contracting the disease during childhood All races seem to be equally liable to infection and statistics show that coloured (negroes, mulattoes) are as susceptible as the white population

A LUTZ and H C DE SOUZA ARAUJO are of the opinion that leprosy can be disseminated by blood-sucking insects, and G M DE OLIVEIRA CASTRO and J MARIANA tried to prove it experimentally by reinfecting bacilli-negative cases by the bites of *Nyssorhynchus albitarsis*, *N strodei* and *Psorophora ciliata* Most leprologists do not accept this theory and are of the opinion that the disease is transmitted by protracted and intimate contact with infectious cases

The typical clinical forms of the disease are seen as Nodular, nervous, tuberculoid and mixed forms I had the opportunity of seeing a number of early infections

Besides chaulmogra, the sulphones (promin, promizole, diazone) have

been used in late years. In nodular forms, and especially in the tuberculoid good results are seen with the sulphones. Aplastic anaemia is relatively rare in *sulphone-treated patients.*

Some of the leper asylums of the country are very modern and perfect. They are built on extensive grounds and the patients occupy themselves—if they want—with agriculture stock raising, or other jobs.

WATER-BORNE DISEASES.

Typhoid, paratyphoid fever dysentery (both bacillary and amoebic) are endemic in the country

The following chart of the mortality coefficients per 100 000 inhabitants in the Brazilian state capitals for the year 1945 illustrates this —

	Typhoid. Paratyphoid.	Dysentery		Typhoid. Paratyphoid.	Dysentery
Aracaju	7·8	21·6	Maceio ..	9·8	145·5
Belem	15 1	37·2	Natal	28·4	65·9
Belo Horizonte	22 7	22·4	Niterol	5·8	17·4
Cuiba	1 7	3·4	Porto Allegre	22 7	22·4
Curitiba	25 7	24·4	Recife	11 5	20·0
Federal District	6 1	10·2	Salvador ...	13·7	6·2
Florianopolis	53 2	20·8	São Luiz	19·3	46·1
Fortaleza	11 7	109·4	São Paulo	5·6	18 1
Goiana	1·9	29·7	Teresinha	113 3	5·7
Joa Pessoa	15 3	71·9	Vittoria	15·5	62·0
Manaos	8·2	86·1			

In rural districts the numbers are even higher

One of the main sanitation problems of the country is to provide towns and rural localities with a pure water supply Federal and State public health services are centralizing their efforts to this end. In the Amazon valley the S.E.S.P service has already built new water plants in some localities, utilizing mostly ground water S.E.S.P is also engaged in building hygienic latrines in these districts.

Typhoid fever has a relatively high mortality ranging for instance in Belo Horizonte between 20 and 30 per cent.

Both forms of dysentery occur in the country Amoebic dysentery is common in its acute and more chronic form. In some Amazonian villages 25 per cent. of the population examined were suffering from the disease. It is amazing that hepatitis and liver abscess are seldom complications of amoebic infection.

VENOMOUS ANIMALS.

Different venomous animals are the cause of morbid symptoms and fatal cases in the country Snakes, scorpions, spiders and some venomous fish are the principal ones. The following snake species are those usually occurring in the country after H. FROES

Conocercae

Glyphodontes — Proteroglyphodontes — Elapidae — Micrurus — M fisheri, M frontalis

Crotalinae

Solenodontes — Crotalidae — Lachesinae

Crotalus — C terrificus (Laur) (*Cascavel*)
Lachesis — L muta (Linn), (*Surucucú*)
Bothrops — B bilineata (Wied)
B jararaca (Wied)
B jararacusu (Lacerda)
B pirajai (Amaral)

In different Institutes the venomous snakes are kept, and horses immunized with the venom for the preparation of monovalent or polyvalent antiserum The best known of these Institutes are the Institute Butantan in São Paulo and the Hygiene Institute (Ezequiol Dias) in Belo Horizonte All chemists by law must keep antiserum and usually the treatment of snake bites is started in time

Scorpion bites are common in some districts, and especially dangerous in children In Belo Horizonte, 1,221 bites by the scorpion, *Tityus*, were registered in 1941 Antiserum is used for treatment, and O MAGELHAES suggests, for heavily infested areas, a prophylactic inoculation for children

This evening I have tried to give you a very brief picture of the many tropical diseases and health problems of Brazil My paper is in no way complete, and I ask you kindly to excuse me if the subjects were not discussed in detail, and nothing new added to the already existing knowledge It was impossible to mention all the tropical diseases and, for instance, plague, ulcus tropicum and others were not discussed

It has been my purpose to give you an impression of the many problems which face the scientists and public health officers of this extended and immense country, and how they are improving the sanitation every year Taking into consideration the numerous endemic tropical diseases and the high infantile mortality, of which the following chart will give you an idea, and which in rural localities is even higher, it is amazing that the Brazilian population, practically without immigration, is yearly increasing by almost 1,000,000 persons

INFANTILE MORTALITY COEFFICIENTS PER 1,000 BIRTHS (LIVING) IN 1945 IN THE BRAZILIAN STATE CAPITALS

Aracaju	343 4	Fortaleza	325 6	Porto Allegre	141 5
Belem	159 6	Goiana	157 7	Salvador	216 2
Belo Horizonte	107 3	Joao Pessoa	229 9	São Luiz	245 9
Cuiba	70 8	Maceio	296 9	São Paulo	101 5
Curitiba	105 6	Manaos	147 7	Terezinha	204 2
Federal District	125 6	Natal	509 7	Vittoria	152 0
Florianopolis	253 7	Niteroi	121 7		

I do not want to finish this paper without saying that everywhere in Brazil I was received with the greatest hospitality and impressed by the wonderful nature of the country HENRY VALLENTON, former Swiss Minister to the

Brazilian Government, rightly gave his book the title "Brésil terre d'amour et de beauté"

Most of the wealth of the country is still undeveloped, and STEFAN ZWEIG, who loved Brazil, called his book "Brésil terre d'avenir"—Brazil, land of the future.

DISCUSSION

The President I think you will agree that we have listened to an extraordinarily interesting and very well illustrated survey of Brazil and its diseases. I cannot pretend to know much about Brazil myself except as an ornithologist. We must congratulate Professor MALAMOS on a remarkable performance. He has given us such a wide survey that it is difficult to pick out any one point for discussion. Some of those here who have experience of the country might like to ask him some questions.

Dr L. E. Napier Dr MALAMOS apologized for mentioning tuberculosis which, he said, is not a tropical disease. But what is a tropical disease? What is a tropical disease today is not one tomorrow and *vice versa*. I think I am in a quite safe position if I say that 100 years hence, perhaps only 50 years hence, tuberculosis will be thought a tropical disease.

Sir George McRobert I agree with Dr NAPIER that Professor MALAMOS need not have apologized for mentioning tuberculosis in front of this Society. Tuberculosis, like typhoid fever and malaria, plays havoc in both temperate and torrid zones. It is most important that young doctors going to the tropics should realize how prevalent tuberculosis is there—either on its own or in conjunction with other conditions. I have heard complaints that candidates appearing for the clinical examination for the D.T.M. & H. have been given non-tropical cases. The examiners were quite right. Pulmonary tuberculosis and rheumatic carditis are too often missed in the tropics and treated by quinine and emetine for very prolonged periods. Professor MALAMOS's observations on the outstanding public health problem presented by tuberculosis in Brazil fall into line with those made in the tropical lands of Asia and Africa.

The man in the street here knows little of Brazil except as the home of coffee and Carmen Miranda, but international statesmen, worried by overcrowding and intolerance in the Old World, have for some time been contemplating the vast green vacuum of South America with longing eyes. We must make sure that they are advised not to submit unsalted and unseasoned innocents to the dire hazards which Professor MALAMOS has revealed to us tonight.

I am greatly interested in the cultural influence exerted by small European nations on larger territories overseas, and I should like to ask Professor MALAMOS if there is much regular intercourse between Brazil and its cultural and linguistic

motherland—Portugal Do the Tropical Diseases institutes of Brazil maintain contacts with Lisbon or have they developed closer affinities with the English-speaking schools of North America, to which Brazil owes so much Incidentally, Londoners must note with envy the advertisements of the Lisbon School of Tropical Medicine in which we read "Adjoining the school is the colonial hospital where clinical demonstrations are given daily" What is impossible on the Thames is evidently feasible on the Tagus!

Col E W Kirwan Dr MALAMOS mentioned the eye I know that trachoma is common in Brazil, and I think the eye diseases in general are similar to those of India I should, however, like to ask about the eye complications in leprosy

Col C H Barber Before the War I happened to be travelling in the East, and found that large numbers of Japanese were being emigrated to Brazil They were going over in shoals at that time, about 1935 or thereabouts I wonder if Dr MALAMOS noticed if they were suffering much from tropical diseases? I did not see any Japanese in his pictures

Dr Malamos (in reply) I would like to thank first of all the speakers who took part in the discussion I agree with both the speakers who said that tuberculosis is a tropical disease and might be sometime the only tropical disease My apologies are due for having mentioned it with an apology In Brazil I think it is a principal problem, and it is difficult to establish methods of combating the disease The Ministry of Education and Public Health have launched a rather large campaign and wish to advance money to the different States so as to establish hospitals for tuberculosis patients, but the States must pay the maintenance cost of the hospitals, and some of the States are poor There is much discussion about the establishment of tuberculosis hospitals The number is insufficient As to displaced persons, Brazil has had a rather stern policy of very small immigration in recent years It seems now that the policy will not be so restrictive There is a similar position in Argentina After the War hundreds of thousands of people, especially Italian, moved into Argentina, and it looks, to begin with, as if no emigrants should go to Brazil That is why I think U N E S C O think of Brazil as a place for the investigation of different problems of natural science As to the Amazon Plain, it may be possible one day to take measures whereby in extensive regions like the Matto Grosso, and elsewhere, millions of people will be able to go there, especially when we can fly I think those measures will be taken Many have been originated, especially by those interests I have mentioned in the paper The great problem today is communications There are a fair number of railways, but they are totally insufficient, and I think the export trade of Brazil would be immensely increased by better communications There was a question about the contact of Brazil and Portugal I think Brazilians like the Portuguese very

much. Hospitals in the north exist for the general public, but Manaos and Belem have Portuguese hospitals for Portuguese who have emigrated to Brazil They maintain hospitals and for a small amount they can have treatment. I do not think that before the War there was much association in a scientific way between Brazil and Portugal. There was much contact with Brazil and France Great Britain and some other States. The younger generation of Brazilians go much to the United States. I went without knowing any Portuguese and I had to rely on French and English. All the people 40 years old or more spoke French, but the younger spoke English or let us say American. Eye complications in leprosy are very numerous. There was a case which I showed I did not mention it especially I think the cases are very similar to those observed in India. A very good book with beautiful coloured plates has been published in Portuguese and English on the complications in leprosy There was a question about the Japanese. There were people native and Portuguese who said that amoebic dysentery was introduced into Brazil by the Japanese During the War they were mostly kept in camps. When I went first to Belem I was astonished to find they had so few vegetables. I asked why and was told We had the Japanese before the War and had plenty of vegetables. But from the time the Japanese colonies disappeared we must bring our vegetables from the south." A pound of vegetables costs much more than a pound of meat I do not think there is any difference in the incidence of tropical diseases between the Brazilian and Japanese population, but I did not have special occasion to make investigations. Again I thank you very much.

The President I think we have had a most successful meeting and are greatly honoured by the presence of His Excellency M LEON V MELAS, Ambassador of Greece, and MADAME MELAS. Dr MALAMOS has covered an immense field, and has had something to say of interest to every one of us. I had never realized that those bromelias, which are a sort of mistletoe were as numerous as he showed them to be. The task of getting rid of them must be enormous. The whole of that part of Brazil is one vast forest so one thinks of the amazing number and variety of the birds, and would like to know how many species of birds, bats and even insects may reside there without ever being seen by civilized man. Dr MALAMOS is entitled to the very high position he has been called upon to occupy in Greece and has now been appointed physician to the Royal Family

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COMMUNICATIONS.

BLOOD EXAMINATION AND PROGNOSIS IN ACUTE FALCIPARUM MALARIA

BY

JOHN W FIELD*
Institute for Medical Research, Malaya

I FOREWORD

Opinion is strangely discordant on the value of blood examination as a guide to the outlook in—even to the presence of—the graver forms of falciparum malaria. Countless observations over a great range of clinical material for over 60 years have failed to settle the simple questions: are the findings of the microscope a sound guide to the gravity of malignant malaria? does pernicious malaria with persistently negative blood films really occur ?

A search through the recent literature of malaria available in this Institute, incomplete from the deficiencies that are an aftermath of the Japanese occupation, illustrates this discordance. LINDSAY (1943), writing from a valley, "the malignancy of whose fever had been noted even in the thirteenth century," takes the view that "the microscope has little place in the diagnosis of pernicious malaria." "A negative blood slide," he states, "has sent many to the grave." Yet HUNTER (1945), from observations on some 3,000 cases of malaria in a forward treatment unit in one of the most malarious areas of the Assam-Burma border, protesting against an uncritical diagnosis of clinical malaria, emphasizes the ease with which parasites are found in serious cases, and seeks

* Some of the cases reviewed in this paper were observed by Dr J H STRAHAN, Dr J F B EDESON or the late Dr J C NIVEN. The writer is indebted to these present or former colleagues in the Malaria Division of the Institute for the use of the clinical records.

to exorcise that "bogey man of the medical officer" the cerebral case of malaria with persistently negative blood slides.

The two views are poles apart. Within this extreme range there is a disposition to accept the evidence of the blood films as reliable in general, but sometimes misleading. KHAN (1945), for example, in a series of 22,041 cases of malaria treated in an Indian general hospital, records 60 deaths from cerebral infection: segmenting parasites were found in the brain of six cases with repeatedly negative blood slides. FITZ HUGH *et al.* (1944) give their experience in a U.S. Army hospital in India where there were 140 cerebral infections in a series of 6,059 cases treated. Some of the deaths, thought to be due to cerebral malaria, were of patients who had few or no demonstrable parasites in blood films. WRIGHT (1941) believes that a sudden onset of coma in acute falciparum malaria, usually caused by an embolism of parasites, may occur when few or no parasites are found in blood films. RANSOME (1944), reviewing the experience of a coma team on the Assam Burma frontier with 170 cases of fully developed malarial coma, mentions that blood slides were apparently negative during coma in three cases proved to be malaria by the autopsy demonstration of parasites in the brain.

Greater confidence in the microscope is expressed by others. LOWE (1944), protesting against the belief that negative films from untreated cases of malaria are quite common, states that he has never seen a case. OGBORN *et al.* (1944) support this view. They saw no serious case of malaria in which parasites were not numerous and one only in a carefully studied series of 396 cases, in which three successive films were negative.

The observations recorded in this paper are presented as a contribution to a problem which is clearly beset with difficulty.

II. MATERIAL STUDIED.

The Malaria Division of this Institute has access to clinical material in the adjoining Government Hospital. In the later years before the war some 3,000 cases of acute malaria of all forms were available for clinical study. With few exceptions they were male adults of Chinese, Indian or Malay race. From this material cases were selected for various forms of experimental treatment. They were chosen, so far as possible, to exclude any likely to show a misleading response to remedies under test. Selection was limited, for example, to persons (i) who had been untreated hitherto, (ii) whose blood showed the presence of a significant number of a single species of malaria parasite, (iii) who had fever at the time of observation, (iv) whose malaria was uncomplicated. These standards were arbitrary. Clinical malaria not confirmed by blood examination was not accepted as malaria—an attitude which had, perhaps, a greater regard for expediency than for truth, but which was, nevertheless, a necessary safeguard against the inclusion of cases masquerading as malaria under the guise of fever, a suggestive history and an enlarged spleen. There was no further

refinement of selection Some cases were primary, some recurrent, some observed early, some late, some were malignant, some clinically benign from long tolerance They represent what is probably a fair cross-section of the acute malaria admitted to the hospitals of this corner of S E Asia over a 10-year period

Some 2,000 cases of acute uncomplicated falciparum malaria selected in this manner were treated in the years before the Japanese invasion, 250 more have been observed since They received test remedies, some good, some bad, but always efficient when infection was severe—usually quinine, atebrin or paludrine Normally, the drugs were given by mouth, sometimes by intramuscular injection, rarely by intravenous injection Fifty-five patients died during treatment Five of the cases were found at autopsy to be complicated The 50 remaining are considered in this paper with special emphasis on the prognostic value of blood examination * The series includes and extends one reported some years ago (FIELD and NIVEN, 1937)

* A broad statistical summary survived the Japanese occupation but not all the case records Clinical analysis of the fatal cases is thus not possible Some were cerebral, some algid, most without localizing signs, seemingly overwhelmed by the parasitaemia

III PROGNOSIS AND PARASITAEMIA IN UNTREATED INFECTION

The peripheral concentration of parasites was determined from thick films made from the capillary blood of the finger Counts were done each day during

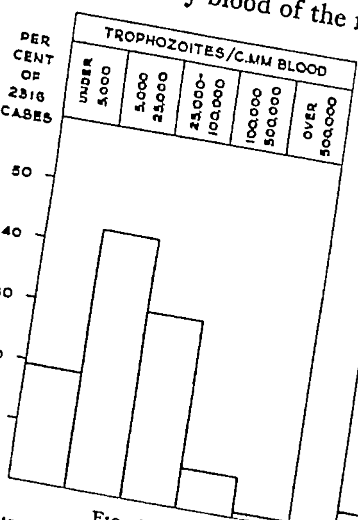

FIG 1
Parasitaemia in 2316 cases of acute falciparum malaria *

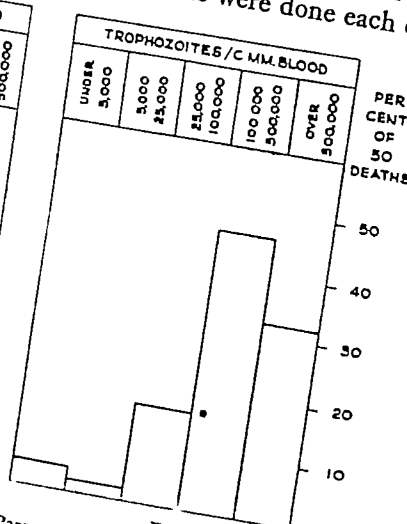

FIG 2
Parasitaemia in 50 fatal cases in the same series †

* This table excludes most cases with parasitaemia less than 1,000 per c.mm These cases are seldom suitable for the evaluation of drugs under test

† Five cases are excluded from this analysis

TST 73/33 Initial count, 2,300 per c mm , autopsy basal pneumon

treatment, the first before treatment started. The DREYER SINTON fowl-cell technique was used throughout.* The degree of parasitaemia in the series, determined by examination of the peripheral blood before treatment began, is analysed crudely in Fig 1

There were 50 deaths in the series, thought to be due solely to the malaria The peripheral concentration of parasites in these fatal cases before treatment began is shown in Fig 2.

Thirty nine of the deaths were in patients whose peripheral blood before treatment showed at least 100,000 parasites per c.mm.† No fatal case was seen in which microscopic diagnosis from a 100-field examination of a thin blood film would be difficult.

Three fatal cases only had peripheral counts less than 10,000 per c.mm. blood—one of 9,500, and two of 2,800 and 2,300 figures which are well above the microscopic threshold for routine thin-film examination.‡

Fig 3 relates the degree of parasitaemia in the fatal cases with the microscopic threshold Fig 4 correlates parasitaemia with the death-rate.

The prognostic significance of parasite counts from blood films in these cases is clear High counts carried high risk, low counts a low one and it seems that death from uncomplicated falciparum malaria among Asiatic adults in this

CST	24/33.	Initial count, 178,000 per c.mm autopsy Shiga dysentery
TST	313/40	Initial count, 36 000 per c.mm. autopsy brain abscess.
QST	238/36	Initial count 60 000 per c.mm. autopsy dysenteric ulceration.
PBT	131/43	Initial count, 2,000 per c.mm ruptured spleen from fall during treatment

* Parasites were usually counted against 100 fowl-cells. Counts were made from thick blood films prepared from a mixture of equal parts of blood and of a suspension containing 10,000 fowl-cells per c.mm. High counts were usually checked from a differential parasite per red cell count in a stained thin-film and a haemocytometer count of the red cells

† Parasite counts may be roughly interpreted in terms of the average number in a field of the microscope Using a 1/12-inch oil immersion lens and a ×8 eyepiece and assuming 200 red cells to the thin-film field, a blood count of 4 000,000 and discounting multiple infection of cells, a parasite count of 100 000 means that one cell in 40 is infected, with five parasites to the thin-film field. The thick-film field would show from 50 to 100 or more parasites. Film thickness varies and red cell counts vary from 1,000,000 to 4 000 000 per c.mm so that the equivalent of counts in parasites per field cannot be given very accurately

‡ The microscopic threshold is that concentration at which parasites are first seen in blood films. It varies widely with the skill and experience of the observer and the time and method of examination. The level given—500 per c.mm. for thin films and 10 to 20 per c.mm. for thick—are arbitrary based on a 100 field examination by a good technician. They assume that the first parasite seen is recognised—not always a fair assumption. But in practice many more than 100 fields would be examined if there were serious doubt.

TABLE I

DAILY TROPHOZOITE COUNTS IN 50 FATAL CASES OF ACUTE FALCIPARUM MALARIA

Case number	Daily trophozoite counts per c.mm. peripheral blood.						
	1	2	3	4	5	6	7
QST 68/35	160,000†						
QST 167/36	300,000	148,000†					
QST 197/36	>500 000	220 000	28,000†				
QST 209/36	540 000	50,000	7 000†				
QST 231/36	82 000	130,000	126 000	2,400	400	0†	
QST 250/36	514 000	750,000	44,000	23 000†			
QST 313/36	162,000	349,000	371,000†				
QST 392/37	350 000	370 000	60 000	4,000	1,200	800†	
QST 396/37	360 000						
QST 402/37	2,800	1,000	<100†				
QST 408/37	770,000	530,000†					
QST 423/37	148 000						
TST 150/37	145,000	30 000†					
TST 212/37	185,000						
TST 227/37	731,000	608,000†					
QST 35/39	310,000†						
QST 100/39	939,000†						
QST 330/39	290 000	109 000	300†				
QST 333/39	233 000	62 000†					
QST 338/39	1,000 000†						
QST 365/39	1,330,000						
QST 362/39	1,200 000	772 000†					
QST 371/40	650 000	773 000†					
TST 311/40	220,000	160,000	68,000	31,000	1,600	100†	
TST 320/40	417,000	270 000	160 000†				
TST 335/40	300 000	120 000	64,000†				
QST 403/40	50,000†						
QST 424/40	124 000†						
QST 430/40	110 000	7,400	<100†				
QST 463/41	305 000†						
QST 464/41	511,000†						
QST 492/41	43,000†						
AST 20/35	108,000	650,000†					
AST 44/35	450,000	300,000	228,000	48,000	2,800	300	0*
AST 45/35	228 000†						
AST 73/35	650,000†						
ACST 4/38	30 000†						
AST 5/38	9,500†						
AMST 2/35	35 000†						
AMST 5/35	700 000	272,000†					
AMST 35/35	>500,000†						
AST 15/47	80,000†						
PST 10/47	274,000	364,000†					
PST 19/47	65,000†						
PST 34/47	61 000	3,000	100†				
PST 78/47	204,000	6,000	500†				
PST 43/47	828 000†						
PST 120/48	426 000†						
PST 131/48	2,300†						
PST 136/48	922,000†						

* Died 22nd day † Died

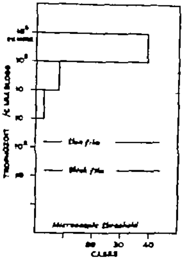

Fig. 3

Parasitaemia before treatment in 50 fatal cases of falciparum malaria in relation to the microscopic threshold.

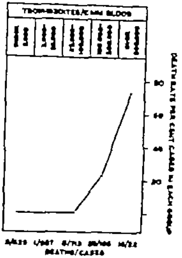

Fig.

Death rate according to parasitaemia (3316 cases, 50 deaths).

particular part of S.E. Asia is rare when parasites are found in blood films and their number while still uninfluenced by treatment, is not great.

IV Prognosis and Parasitaemia in Treated Infection.

Prognosis from blood films becomes less reliable when parasitaemia is reduced by treatment. Death may occur when the numbers have fallen to a level at which parasites are not found by routine thick film examination and it is by no means a rare event for a patient to pass into coma when the numbers are falling rapidly 24 or even 48 hours after the start of treatment. The daily counts up till the day of death in the 50 fatal cases are summarized in Table I. Most cases still showed high parasitaemia on the day of death but not all. Six cases gave counts shortly before death at which thin-film recognition might conceivably have been difficult two cases were parasite-free on the sixth and eighth day of treatment. How far this fall in parasitaemia to a level at or below the microscopic threshold for thin films can explain that bogey man of the medical officer the cerebral case of malaria with persistently negative blood slides is uncertain. Not all cases coming under medical observation for the first time are likely to have arrived untreated.

It is perhaps well to emphasize that treatment, while reducing the prognostic value of blood films, does not necessarily obscure diagnosis. Many cases can still be diagnosed as malaria while the fever lasts and though treatment produces a quick fall, the numbers of parasites tend to remain above the microscopic

threshold for a period which depends on the activity of the drug used Diagnosis is not strictly relevant to the subject of this paper but the diagnostic reliability of blood films during treatment is so important clinically that evidence available from this series may, perhaps, be considered briefly

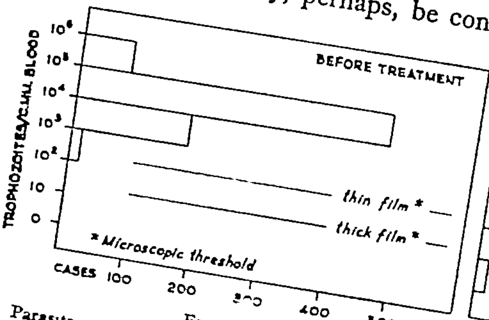

FIG 5
Parasitaemia before treatment in 736 quinine treated cases of acute falciparum malaria considered in relation to the microscopic threshold.

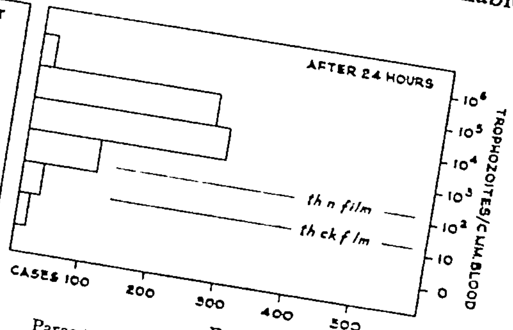

FIG 6
Parasitaemia in the same series 24 hours after starting treatment.

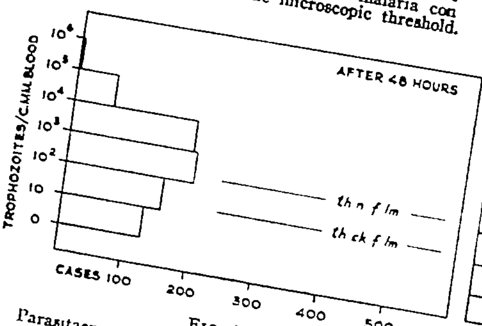

FIG 7
Parasitaemia in the same series 48 hours after starting treatment.

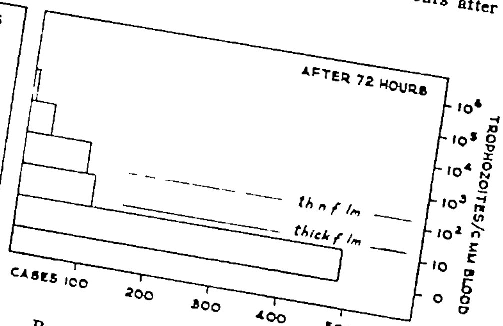

FIG 8
Parasitaemia in the same series 72 hours after starting treatment

Figs 5 to 8 present an analysis of the parasite counts in 736 cases treated with quinine It may be observed that diagnosis from blood films would still have been possible in most cases after 24 hours' treatment, and in some after 48 or even 72 hours

V PROGNOSIS AND THE PRESENCE OF SCHIZONTS IN BLOOD FILMS

Peripheral schizogony in falciparum malaria is commonly regarded as a grave event RAPER *et al* (1945) doubt whether this assumption is always true They found schizonts in the peripheral blood of African natives who were not very ill Maybe, they suggest, there are strains of *Plasmodium falciparum* which more nearly follow the pattern of asexual distribution of other species ; or maybe the African has an overworked and partly blocked reticulo-endothelial system which allows schizonts to overflow into the peripheral blood more freely than is usual

Schizonts were found in thick blood films examined before treatment in 79 cases in this series. The schizont incidence at the differing levels of parasitaemia is shown in Fig. 9. Figs. 10 to 12 relate the presence of schizonts with the chances of recovery.

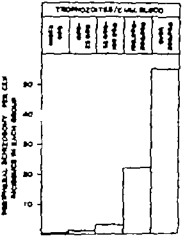

Fig. 9
Schizont incidence related to parasitaemia.

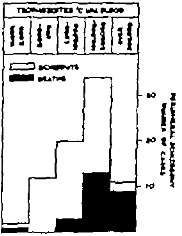

Fig. 10
Distribution of 36 deaths related to parasitaemia among 79 cases showing peripheral schizogony

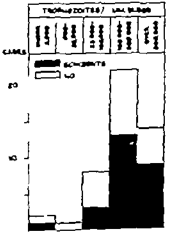

Fig. 11
Peripheral schizogony on first examination in 47 fatal cases.

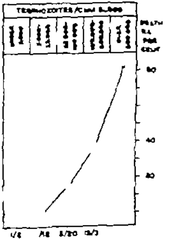

Fig. 12
Death rate at the different levels of parasitaemia in 79 cases showing peripheral schizogony on first examination

No record survives of the presence or absence of peripheral schizogony in three fatal cases omitted from this analysis.

The well-known tendency for peripheral schizogony to vary with the degree of parasitaemia is well supported by Fig 9 The presence of schizonts in blood films, however, seemed to bear little relation to the death-rate at the lower levels of parasitaemia. Thirteen patients recovered of 14 with peripheral schizogony on first examination and counts below 25,000 per c mm, four died of 34 with initial counts lower than 100,000 This was the only evidence suggesting that the presence of schizonts in blood films, considered apart from the parasite count, had much prognostic significance (cf Fig 4)

There were 21 fatal cases in which schizonts were not seen in blood films on first examination (Table II)

TABLE II

PARASITAEMIA IN 21 FATAL CASES OF ACUTE FALCIPARUM MALARIA IN WHICH SCHIZONTS WERE NOT SEEN ON FIRST EXAMINATION

Serial number	Initial parasite count per c.mm. peripheral blood	Serial number	Initial parasite count per c.mm. peripheral blood.
QST 167/36	300,000	QST 430/40	110,000
QST 209/36	540,000	QST 371/40	556,000
QST 231/36	82,000	TST 335/40	306,000
QST 313/36	162,000	QST 464/41	511,000
QST 402/37	2,800	AST 26/35	108,000
TST 150/37	145,000	AST 44/35	450,000
QST 190/39	940,000	ACST 4/38	30,000
QST 330/39	290,000	AST 5/38	9,500
QST 333/39	233,000	AMST 2/35	35,000
QST 338/39	1,000,000	AST 15/47	80,000
QST 403/40	50,000		

There is thus clear evidence here that the absence of schizonts from blood films was not necessarily a favourable prognostic sign, nor, on the contrary, was their presence, considered apart from the degree of parasitaemia, necessarily unfavourable, and it seems fair to conclude that peripheral schizogony in this particular series had less prognostic value than the peripheral concentration of parasites

VI PERNICIOUS MALARIA WITH FEW OR NO PARASITES IN BLOOD FILMS

(a) *Intermittency in Peripheral Parasitaemia*

Schizogony in falciparum malaria normally occurs in the capillaries of the inner organs The ring forms disappear from the peripheral circulation when about 18 hours old It is thought that the infected cells become sticky and clump together or adhere to large mononuclear cells to form emboli which are

held up in the capillaries of the brain, liver spleen, bone-marrow and other inner organs or tissues. The cycle lasts about 48 hours. There is thus a period of some 30 hours when the older forms of any particular brood are absent from the peripheral blood. At the lower levels of parasite concentration this phenomenon is not rare Parasites seen one day are absent the next, to appear again on the third day In other words, the synchronous development of a single brood may lead to confusion in diagnosis if not in prognosis during a temporary lull in the parasitaemia.

The parasite counts in this series have been examined with this possibility in mind. There is no record of a single severe infection in which the synchronous development of a single brood was so uniform that the parasites vanished from blood films to return in big numbers the next day The greater the parasitaemia, it seems, the more it is likely that there are several or many broods at differing stages of growth. There is normally a multi-brood development in falciparum infection. Ring forms recede to the inner organs to be replaced by others which are younger and the peripheral parasitaemia, though subject to fluctuation from this ebb and flow tends to a fairly steady decline with treatment or increase without. Fluctuation is usual but, to use a metaphor it is a surface fluctuation. When the parasite reservoir is shallow—early in the attack or with low-grade infection—the swing is maximal and parasites may appear and disappear from blood films on successive days but when deep with infection heavy enough to be serious, fluctuation is likely to be no more than the merest ripple on the surface Exceptions to this general rule observed in the present series are recorded in Table III.

Table III records the daily counts in cases with a large dominant brood These were the only cases, of 177 having initial counts of 100,000 per c.mm. or more, in which a fluctuation in numbers might possibly have been misleading Blood examination on successive days might fairly have been expected to remove any doubt arising from a single examination.

With one exception these patients recovered. The fatal case AST 26/35 has a special interest. The outlook, judged from the first blood film, was favourable 24 hours later it was almost hopeless—a departure from the general rule that blood examination gave a better indication of severity before treatment than later on.

(b) Grave Infection with few Peripheral Parasites

Perhaps the commonest source of confusion in malaria diagnosis is the assumption that the presence in the peripheral blood of a few falciparum rings is necessarily related to the associated symptoms The perplexed physician

Dr T Wilson informs the writer that infections of great intensity almost, but not entirely restricted to single brood occurred among European and Australian prisoners of war in Singapore and Siam during the Japanese occupation. Parasitaemia was recorded as heavy very light and heavy on successive days.

TABLE III

DAILY PARASITE COUNTS IN CASES, WITH INITIAL PARASITAEMIA OF 100,000 PER C MM OR MORE SHOWING SIGNIFICANT INTERMITTENCY IN NUMBERS

Case		Daily trophozoite counts per c.mm blood						
		Day 1	2	3	4	5	6	7
AST	26/35	108,000	659,000*					
TST	70/33	370,000	500	103,000	1,200	50	0	0
CST	56/33	144,000	2,800	26,000	500	0	0	0
AMST	84/35	100,000	0	200	0	0	0	0
AST	56/35	562,000	4,800	54,000	3,000	<100	0	0
APLASST	24/38	220,000	4 800	22,000	11,000	<100	0	0
QST	401/40	200,000	15,000	100,000	11,000	700	0	0
TST	263/40	124,000	<100	16,500	6,000	0	0	0
TST	309/40	104,000	600	13,600	100	0	0	0
AST	13/40	134,000	500	22,000	700	0	0	0
AST	18/40	144,000	4,000	32,000	3,000	<100	0	0

* Case AST 26/35

	Day 1	2
Trophozoites	108,000	659 000
Schizonts	0	28 000

faced with a laboratory report of parasites in blood films can be forgiven a tendency to assume that they are causally related to the symptoms if he can find no other explanation Usually, he will be right, but sometimes wrong, as is clearly shown by the recent clinical and laboratory study of OGBORN and his colleagues (1944) These workers found that of 512 African natives admitted to hospital with malarial parasites in the blood, 116 were suffering from relapsing fever, typhoid, dysentery, acute respiratory infection or other diseases responsible for the symptoms

Does the parasitaemia in grave falciparum infection ever stay persistently low? Are there pernicious cases which, untreated, never show more than a few rings in serial blood films? This is a problem of great clinical importance for which the evidence from this series has no clear answer The cases here recorded were treated Low parasitaemia in clinically grave infection was due to treatment—with three exceptions There was an initial parasitaemia in three fatal cases of 2,300, 2,800 and 9,500 per c mm Two of these patients died within 24 hours

Two of these cases are recorded as falciparum deaths in the absence of a clear alternative diagnosis, the third, despite the presence of gangrene of the lung, because the blood and brain contained falciparum schizonts The evidence they give of uncomplicated pernicious infection with low parasitaemia is unsatisfactory There is no definite autopsy proof in two that malaria was the cause

of death. They were the only fatal cases, among 1 426 falciparum infections, with initial parasite counts below 25,000 per c.mm. which might be considered to support the belief that *Plasmodium falciparum* may cause death without reaching the peripheral blood in significant numbers.

TABLE IV

FATAL FALCIPARUM INFECTION WITH LOW PARASITAEMIA.

Case.	Trophozoites per c.mm.			Comment.
	Day 1	2	3	
QST 402/37	2,000	1,000	<100†	Died suddenly on 3rd day of quinine treatment. No parasites in brain at autopsy. Immediate cause of death haemorrhage into alveoli of lung.
AST 8/38	9,600†			No surviving autopsy record.
PST 131/48	2,300†			Schizonts in blood film on first examination. Autopsy schizonts in brain; gangrene of lung

(c) *Persistent Absence of Peripheral Parasites.*

No diagnosis of pernicious malaria in the persistent absence of parasites in blood films is valid, in the view of the writer without the demonstration at autopsy of parasites in vital inner organs. Seldom is this proof given, though RANSOME (1944) and KHAN (1945) found falciparum schizonts in the brain when they had been unable to find parasites in blood films. There is no direct evidence from the present series on this important question, since by definition the series consists only of cases with parasites in blood films but the rarity of clinically grave infection with low initial parasitaemia at least suggests that serious infection with no peripheral parasites is similarly rare though not excluded. What, then, should be done when pernicious infection is thought to be a possibility remote perhaps but grim, and no peripheral parasites are seen to support this suspicion? Does clinical suspicion without laboratory confirmation justify the urgent attention which pernicious malaria demands? None will dispute here the attitude of NELSON-JONES (1944) that no patient should ever be allowed to "die of untreated malaria because the disease appeared in an atypical form with negative blood slides." Clinical judgment suspended from a doctrinaire assurance that parasites always invade the peripheral blood stream when infection is severe may cost life and most will probably agree with his insistence on immediate diagnosis and treatment when there is

† Two cases with low initial counts, PST 73/33 and PST 131/48, are excluded One died from pneumonia and the second from rupture of the spleen.

adequate clinical evidence, without waiting for positive results from blood slides " Yet, sound though this attitude may be, it should not mislead the physician to a belief that every case treated as malignant malaria and the subject recovering after a few injections of quinine, or dying without autopsy proof of the cause, is necessarily correctly diagnosed A diagnosis which may be clinically expedient is not necessarily valid evidence

Confusion may arise from

(i) The simulation of cerebral malaria by other diseases, virulent pneumonia, typhoid fever, tuberculous meningitis, head injury, acute alcoholism, for example, may all produce symptoms similar to those sometimes seen in cerebral malaria (WRIGHT, 1945)

(ii) The failure to recognise peripheral parasites The Romanowsky stains on which we rely almost wholely today for malarial diagnosis are subject to strange vagaries in the hands of workers who are not using them so often as to understand their occasional capricious behaviour Most workers in malaria have seen examples of missed heavy infection attributable to defective stains, poor technique or inexperience

(iii) Previous treatment

It is difficult on present evidence to decide how far these sources of error have led to the view that serious, even fatal, malaria can occur without parasites ever reaching the peripheral blood The possibility cannot be denied but, in the view of the writer, no evidence is valid which does not meet the demands that

(i) Other possible causes of the symptoms are excluded
(ii) The parasites have not receded from the peripheral blood as a result of earlier treatment
(iii) The staining is reliable
(iv) Parasites are found at autopsy in vital organs

When this evidence is forthcoming, not from one territory only but from different parts of the world, the vexed question of blood-negative pernicious malaria may perhaps finally be settled But not until then

VII BEYOND WHAT LIMIT OF PERIPHERAL PARASITAEMIA IS FALCIPARUM MALARIA NECESSARILY FATAL ?

Details of an exceptionally heavy infection were reported from this Institute a few years ago (FIELD, 1937) An adult Indian male with a peripheral parasitaemia of 662,000 per c mm made a complete recovery The case was not unique, but this peripheral count was then the highest with recovery observed in this Institute Little seemed to be known of parasite intensity in non-fatal infection and the suggestion was made that three-quarters of a million parasites per c mm of peripheral blood was perhaps the upper limit with recovery That figure has not been exceeded in any of the thousand or so non-fatal falciparum infections seen here since

Six patients in the series survived infections with a parasitaemia of 500,000

per c.mm. or more. The daily counts from five of them are placed on record counts from the sixth case are lost.

TABLE V

DAILY PARASITE COUNTS IN FIVE PATIENTS SURVIVING FALCIPARUM PARASITAEMIA OF 500 000 PER C.MM. OR MORE.

Case.	Trophozoites per c.mm.							
	Day 1	2	3	4	5	6	7	10
AMST 182/36	84,000	250,000	250,000	662,000	414,000	170 000	6 000	0
QST 420/37	640,000	31,000	31,000	400	0	0	0	—
QST 473/41	622,000	340,000	26 000	1,200	<100	0	0	—
QST 1/47	630 000	180 000	2,000	<100	0	0	0	—
PST 24/47	530 000	294 000	7 000	<100	0	0	0	—

TABLE VI.

PERCENTAGE OF INFECTED CELLS IN FIVE PATIENTS SURVIVING FALCIPARUM PARASITAEMIA OF 500,000 PER C.MM. OR MORE.

Case.	Percentage parasites per red cells.	Percentage infected cells.
AMST 182/36	18	18
QST 420/37	16	14
QST 473/41	23	21
QST 1/47	14	—
PST 24/47	18	—

Dow and MEYER (1944) report recovery from falciparum infection in which 34 per cent. of the red cells contained parasites.

TABLE VII.

HIGH PERIPHERAL PARASITAEMIA WITH RECOVERY (STRAHAN, 1948).

Number	Peripheral concentration of parasites per c.mm. blood.						
	Day 1	2	3	4	5	6	7
8	871 000	800,000	275,000	+++	±	±	
10	825,000	725 000	162,000	+++	±	±	0
13†	1,240,000	840,000	135,000	41 400	800	0	0†

† Died on the 21st day from pyaemia due to septic infection at the site of the drip.

STRAHAN (1948) supplements this information from his experience in risoner-of-war camps in Singapore where severe falciparum infections were ommon Using a continuous-drip method of intravenous quinine treatment, e observed recovery from infection for which, from pre-war experience among siatics in Malaya, a fatal prognosis would formerly have been given with omplete assurance The records in Table VII are taken from STRAHAN'S aper

Intravenous quinine given continuously by a saline-drip technique for 48 ours or more gave better results in STRAHAN'S hands than any method known o his colleagues before the war The outlook for these extremely heavy infecions may now, it seems, be brighter than the material analysed in this paper vould appear to indicate

COMMENT

The experience of the Malaria Division of this Institute has so clearly ointed to the superiority of blood examination, and particularly of parasite ounts, over clinical judgment in most cases of falciparum malaria, not only for liagnosis—few will deny this—but also as a guide to the chances of recovery, hat the writer advocates the adoption of routine counts in all serious cases Made before the issue is clouded by treatment, they give information which can e got in no other way They are, he believes, the most reliable indication of everity in all but very few cases The technique is simple a count of parasites er 1,000 red cells in a Romanowsky-stained thin film, and a total red cell ount With this information the clinician possesses a valuable aid to clinical udgment He has also a useful yardstick by which severity in different cases or in different parts of the world can be assessed, and a measurement from which the limiting intensity of recoverable infection might ultimately be determined

SUMMARY

In a series of 2,316 cases of acute uncomplicated falciparum malaria, there were 50 deaths Parasites in blood films were counted in all cases daily during treatment Analysis of this material shows a close relation between the death-rate and the peripheral concentration of parasites at the time treatment began Parasite counts made at this time were more reliable than clinical judgment as a guide to prognosis, and their routine adoption in all serious falciparum infections is recommended

With few exceptions the presence of falciparum schizonts in blood films at the lower levels of parasitaemia was not a reliable indication of clinical gravity

The vexed question of blood-negative pernicious malaria is briefly discussed

Evidence on the limiting intensity of non-fatal falciparum infection is given.

REFERENCES.

DON, C. S. O. & MEYER, P. F. (1944). *Brit. Med. J.* 29th Jan., p. 149 (*Trop. Dis. Bull.* (1944) 41 358.)
FIELD, J. W., & NIVEN, J. C. (1937). *Trans. R. Soc. trop. Med. Hyg.* 30 560
——— (1937). *Ibid.*, 30 565.
FITZ-HUGH, T., JR., PEPPER, D. S. & HOPKINS, H. U. (1944). *Bull. U.S. Army Med. Dept.*, No 83, 39 (*Trop. Dis. Bull.* (1945), 42, 341.)
HUNTER, T. A. A. (1945). *Indian Med. Gaz.* 80 247 (*Trop. Dis. Bull.* (1946) 43, 293.)
KHAN, N. U. (1945). *J. Roy. Army Med. Corps.*, 84 263. (*Trop. Dis. Bull.* (1945), 42, 861.)
LINDSAY, D. K. (1943). *Trans. R. Soc. trop. Med. Hyg.*, 37 63.
LOWE, J. (1944) *Indian Med. Gaz.* 79 207 (*Trop. Dis. Bull.* (1944), 41 997.)
NELSON-JONES, A. (1944). *Ann. trop. Med. Parasit.* 38, 1
OSBORNE, R. S., RAPER, A. B., & WRIGHT, R. J. (1944). *East African Med. J.* 21 101
RANSOME, — (1944). Personal communication.
RAPER, A. B., WILSON, MARGARET E., & WILSON, D. B. (1945). *Trans. R. Soc. trop. Med. Hyg.* 38 291
STRAHAN, J. H. (1943). *Ibid.* 31 669
WRIGHT, F. S. (1941). *East African Med. J.* 18, 226 (*Trop. Dis. Bull.* (1942) 39 432.)

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THE MORPHOLOGY OF MALARIA PARASITES IN THICK BLOOD FILMS

THE FORM AND DISTRIBUTION OF PIGMENT—*Part V* *

BY

JOHN W FIELD,

From the Institute for Medical Research, Kuala Lumpur, Malaya

When malaria parasites grow at the expense of cells containing haemoglobin they produce as a metabolic by-product an iron-containing pigment chemically related to haematin Minute granules of the pigment appear at about the time when the young trophozoite begins to lose its vacuole The granules grow bigger as the parasite grows, then they concentrate or coalesce or scatter They assume distinctive patterns This paper describes these patterns and suggests how they may be used as an aid to the diagnosis of parasite species and phase

SOME GENERAL REMARKS ON MALARIA PIGMENT

Malaria pigment is variously described as yellow, yellow-green, golden-brown and is often shown in colour drawings of parasites as black The differences are related less to the physical characters of the pigment than to

* The earlier papers appeared in the *Transactions of the Royal Society of Tropical Medicine and Hygiene* before the war Part 1, 32, 457, Part 2, 33, 507, Part 3, 34, 297, Part 4, 34, 405

the conditions under which it is examined. The colour impression received by the eye is modified, for example, by

( ) the colour resolution of the optical system ;
(ii) the intensity of the light by which the pigment is examined
(iii) the spectrum transmission of the filter if the light is artificial
(iv) the accuracy of focus of the microscope condenser
( ) the degree of concentration or coalescence of the pigment granules
(vi) the degree to which the pigment is obscured by blue-stained cytoplasm.

Most immersion lenses are well enough corrected to show the colour of malaria pigment and, if colour defects there are, they are more likely to be due to maladjustment of the microscope than to defects in the lenses. Probably the commonest error is a badly adjusted condenser Defects of focusing and centring impair the definition of the smaller granules of pigment and darken the colour—a fact which can easily be verified by racking down the condenser from a position of accurate focus and observing the progressive blackening of the pigment and the development of a hazy fringe around the granules.

The importance of the composition and intensity of the light needs no emphasis. The brighter the light the brighter the colour of the pigment a poor light shows the granules as nearly black. Important, too, is the composition of the light. Unfiltered artificial light, with its dominence of red and yellow rays, shows the pigment as light yellow filtration with a strong blue filter renders it almost black.

The depth of staining of the cytoplasm also has a marked effect on colour and definition. Granules which shine clear and yellow through pale-stained cytoplasm are degraded to a greenish blue with deep staining and may be obscured completely

Finally there is the concentration factor Malaria pigment varies in form from minute granules scarcely visible, to compact masses 2μ or more in diameter Concentration leads to darkening The faint pigment haze which tinges the cytoplasm of the growing trophozoite is, for example, much lighter than the same pigment concentrated and fused in the mature schizont to an almost black mass.

Pigment formation is first visible at the later "ring stage before the cytoplasm begins to envelop the chromatin bead The granules are then very small and cannot as a rule be defined individually except n unstained films. With further growth the separate granules become visible at first small, but growing larger as the parasite grows. Thereafter the pigment of the schizogonic forms begins to concentrate. Concentration begins earliest and is most complet with *Plasmodium falciparum* it begins latest and is least marked with *P malariae* The pigment of gametocytes does not concentrate, except in mature falciparum gametocytes. It remains scattered with tendency to peripheral rather than central distribution.

The pigment grains themselves, too, have tendencies which are specifically distinctive The coarse rods of pigment in falciparum gametocytes, the finer and shorter rodlets of vivax pigment, and the rounded granular formation of quartan pigment are often distinctive enough to give a clue to species

Familiarity with these tendencies is a useful aid to species diagnosis in thick films The presence of pigment in parasites beyond the early trophozoite stage is extraordinarily constant, there are few artefacts in Romanowsky-stained films with which it can be confused, and the story it has to tell of the presence of malaria is so unequivocal that a detailed study of its form and distribution in each species cannot fail to contribute to diagnostic accuracy Before the war, while the material for this study was being assembled, the writer examined a series of lysed unstained thick films from 500 cases of malaria Diagnosis was made in the first place solely from the pigment, and checked later from stained films The records are lost, but he recalls that once pigment had been formed a diagnosis of malaria, and even of species and phase, could usually be made without difficulty from the pigment alone

Malaria pigment is best seen in unstained films The size, form and arrangement of the granules—the main characteristics assisting the recognition of species and phase—are seen more clearly in films which have lost most of their haemoglobin but are not stained, and it is on the lysed but unstained thick film that this study is based Staining does not change the size, shape or disposition of the pigment it merely veils the definition, and if the observer has a clear mental image of the appearance to be expected with each species at each stage of growth he will find it easier to interpret what he sees through the veil of stained cytoplasm *

The Pigment of *P falciparum*

Fig 1 —The pigment of the asexual forms of *P falciparum* in lysed thick blood films

Figure 1A —Pigment first appears as half a dozen or so minute granules when the diameter of the parasite is about half that of the host cell, a few hours before the infected cells normally recede to the inner organs and disappear from the peripheral blood The granules are easily seen in unstained material but in stained films show only as a greenish haze within the cytoplasm The tracing shows the pigment from 15 " rings " The diagnostic value of pigment at this stage is small

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* All drawings of pigment are camera lucida tracings from lysed unstained thick films The drawings of parasites within the host cell which are the key to the phase of growth are tracing from fixed, Giemsa-stained thin films The optical system was a binocular microscope, a 1/16 inch immersion lens and × 10 eyepieces

Lysed thick blood films suitable for a study of malaria pigment are conveniently made as follows (i) prepare a blood film of such a thickness that the hands of a watch can be seen through it and let it stand until it appears to be dry, (ii) dip the film for a second or so into clean water, (iii) place the slide on end to draw and dry, (iv) make a mark with a lead or grease pencil across the film to help focus when the film is examined

There is partial lysis and most of the haemoglobin drains down the slide Examined with an oil immersion lens there is a clean uniform yellowish background with rounded pallid areas which mark the leucocytes Malaria pigment is well seen on this ground of residual haemoglobin

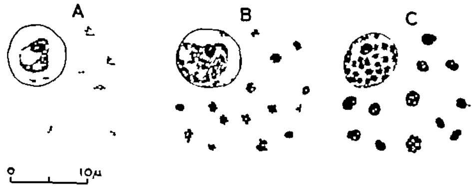

FIG. 1

FIGURE 1B.—With early schizogony there is an increase in size of the granules with very early concentration and coalescence. Concentration is here complete and coalescence of the granules is beginning. The tracing shows the pigment from 14 half-grown schizonts. Pigment is now useful aid to the recognition of species and phase.

FIGURE 1C.—The pigment of the mature schizont is typically single, dark, rounded, solid-looking mass of unmistakable identity. No other species at any phase shows much solid fusion.

FIGURE 2A.—This is the pigment of the ery young gametocyte which is still intra cellular. The granules are typically rather coarse short rodlets, the shape of rice grain. They are scattered and have suggestion of linear distribution which corresponds to the shape of the parasite. The tracings show the pigment from seven young gametocytes.

FIG. 2.—The pigment of the gametocytes of *P. falciparum* in lysed thick blood films

FIGURE 2B.—The older gametocyte, still young, which is extracellular and tends t have rather pointed ends—the cigar form. The coarse rice-grain-like rodlets are still scattered and still have some tendency to linear arrangement. The tracings are from fi such gametocytes. Pigment of these young gametocytes (2A and 2B) can often, but not always be identified by the coarseness and shape of the granules and the manner of distribution. Such pigment is likely t be associated with heavy infection and is seldom of much diagnostic aid.

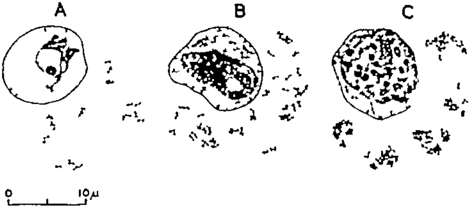

FIG. 4

FIGURE 4B.—The granules are now larger and there are more of them. They are typically short rather delicate rodlets irregularly scattered. Their diagnostic value lies mainly in the assistance they give in the differentiation between vivax trophozoites and artifacts from chance combinations of platelet and chromatoid debris. Four collections of granules are shown.

FIGURE 4C.—Concentration of the granules has now begun. The small short rodlet are fairly distinctive. Most of them are still discrete; there is little coalescence. The pigment may be confused with that of *P. malariae* at a similar stage.

FIGURE 5A.—Concentration of the pigment is now maximal. Some mature schizonts have a single compact collection of discrete granules; some have a slight peripheral scatter. Coalescence of the granules is unusual. Differentiation from the pigment of *P. malariae* at the same stage may be difficult.

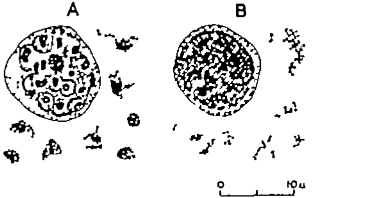

FIG. 5.—The pigment of ripe vivax segmenters and of vivax gametocytes in lysed thick blood films.

FIGURE 5B.—The granules of pigment in the vivax gametocyte have typically a wide and irregular scatter. There is no concentration and no coalescence. Distinction from the pigment of late vivax trophozoite may be difficult. No distinctive differences have been observed. The isolated pigment from three parasites is shown.

The Pigment of *P malariae*

Figure 6A —Pigment formation begins very early Rings of the size shown already have a collection of half a dozen to a dozen small granules This early appearance is sometimes a diagnostic aid Twelve such collections are drawn

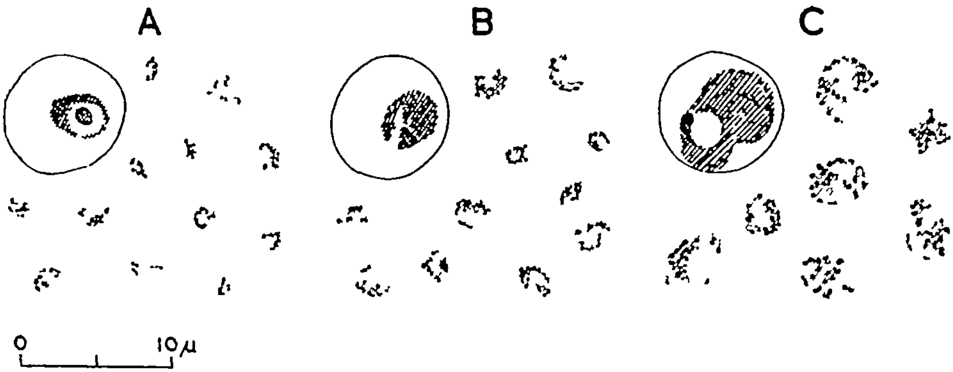

Fig 6—The pigment of the trophozoites of *P malariae* in lysed thick blood films

Figure 6B —The collection of pigment granules remains fairly compact, as, in fact, is the parasite itself There is a tendency to a peripheral distribution with little peripheral scatter

Figure 6C —The peripheral tendency remains The collections are now larger, so are the granules There is often a rough circular or oval arrangement of the granule clusters The granules themselves are correctly so described They lack the slight elongation to rodlet form which is usual with the pigment of *P vivax* and of *P falciparum* gametocytes

Figure 7A —Concentration of granules in some parasites begins with schizogony but dispersal is common Concentration begins latest with *P malariae* and remains least evident The "granule" rather than "rodlet" tendency is well shown

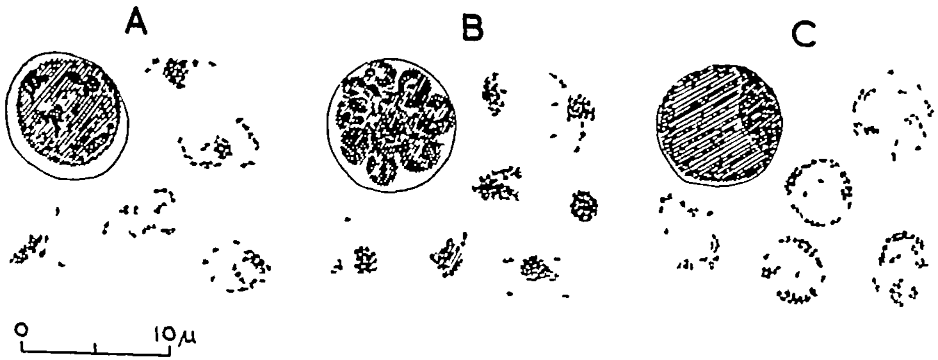

Fig 7—The pigment of the schizonts and gametocytes of *P malariae* in lysed thick blood films

FIGURE 7B.—Concentration is maximal in the ripe schizont. Some parasites still show peripheral scatter of the granules or radial dispersion between the merozoites. Others have a single compact collection of granules. Coalescence of the granules into masses is unusual. Differentiation from the pigment of *P. vivax* at this stage may be difficult.

FIGURE 7C.—The granules in the gametocyte are scattered with special tendency to a peripheral arrangement, and a circular or oval form which corresponds to the shape and size of the parasite. The late trophozoite has somewhat similar arrangement. It is doubtful whether the gametocyte is more heavily pigmented than that of *P. vivax*, but the collection of granules is somewhat tighter. No distinctive sex differences have been observed.

SUMMARY OF CONTENTS.

The paper describes and illustrates the form of malaria pigment in lysed, thick blood films and suggests how the patterns of the pigment may aid the diagnosis of malaria and the identification of species and phase.

The camera lucida tracings made before the war were redrawn in form suitable for reproduction by Mr. YAP LOY FONG, whose help is gratefully acknowledged.

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IN VITRO EFFECTS OF CHLOROMYCETIN ON MALAYAN BACTERIA

BY

R GREEN, M D , D SC ,

AND

D S MANKIKAR, M B , B S

From the Institute for Medical Research, Kuala Lumpur, Malaya

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The object of this paper is to define tentatively some of the further clinical uses of chloromycetin—in accordance with its observed effects on Malayan pathogenic bacteria

Chloromycetin, an antibiotic obtained from a *Streptomyces* (EHRLICH *et al*, 1947) inhibits the growth of certain gram-positive and gram-negative bacteria (SMITH *et al*, 1948) It is also known to affect *Rickettsia* and two viruses of the psittacosis-lymphogranuloma venereum group (SMADEL and JACKSON, 1947, 1948) An alternative name for this antibiotic is chloramphenicol, and it can now be made synthetically Chloromycetin is stable, relatively non-toxic, and when given by mouth is able to attain the comparatively high concentration in the blood of 20 to 80 μg per ml ,* reaching within 2

* For comparison, concentrations of other antibiotics, as usually obtained in the blood, are shown as follows (in units or μg per ml) —

Penicillin (2,000,000 units daily) 1 5 to 2 units KOLMER (1947)
Streptomycin (0 3 gramme 3-hourly) 9 to 10 μg KOLMER (1947)
Aerosporin (0 4 to 0 8 mg per kg , 4-hourly) 0 2 to 0 4 μg SWIFT (1948)
Polymyxin D (0 3 to 0 4 mg per kg , 3-hourly) 0 6 μg SCHOENBACH *et al* (1948)
Aureomycin (1 gramme by mouth 6-hourly) 2 μg FINLAND *et al* (1948)

Notes —One unit of penicillin = 0 6 μg
Aerosporin (polymyxin A) and polymyxin D are similar but not identical in origin and effects (COLLIER, 1948)

hours a tenfold or even greater concentration in the urine (LEY *et al.* 1948, WOODWARD *et al.*, 1948). According to SMADEL *et al.* (1949), the amount of chloromycetin in the cerebrospinal fluid approximates to about one half that of the blood.

Bacteria in the various body fluids and tissues are thus believed to be subject to varying amounts of chloromycetin up to 80 µg. per ml. with a corresponding tenfold or stronger surface effect on the epithelium of the genito-urinary tract.

In Malaya, chloromycetin has been used successfully in treating scrub typhus (SMADEL *et al.*, 1948) and typhoid fever (WOODWARD *et al.* 1948). Following the course of these clinical trials, the writers had the opportunity of investigating the *in vitro* effects of chloromycetin on a series of Malayan disease-causing bacteria. (Experimental quantities of the antibiotic were kindly provided by Dr. J. E. SMADEL.)

These pathogenic bacteria included the organisms of the enteric fevers and of dysentery, cholera, meningitis, melioidosis, pyelitis, diphtheria, gonorrhoea, pneumonia, wound infections, etc.

The technique of testing susceptibility to the antibiotic was mainly the ring-cup method, varying amounts of chloromycetin being placed within 15 mm. glass cylinders sealed on to the surface of suitable culture plates which were either "seeded" or streaked with the bacterium to be treated. Special methods were used for more delicate organisms, e.g. *H. influenzae*, *N. gonorrhoeae* and *Str. pneumoniae*.

Malayan bacteria so far tested by the writers and found to be inhibited by 40 to 60 µg. of chloromycetin are shown in the following table.

TABLE

MALAYAN BACTERIA INHIBITED BY 40 TO 60 µG. OF CHLOROMYCETIN

Bacterium.	Characteristic disease produced.	Bacterium.	Characteristic disease produced.
Salm. typhi	Typhoid fever	*C. diphtheriae*	Diphtheria
paratyphi A, B	Paratyphoid fever	*Bact. friedländeri*	Pneumonia
		Str. pneumoniae	
sp.	Fevers, etc. (see notes)	*H. influenzae*	Meningitis
		Strep. pyogenes	Wound infections, etc.
		Strep. viridans	(see notes)
Shigella sonnei	Dysentery	*Strep. faecalis*	Pyelitis
Vib. cholerae	Cholera	*Bact. coli*	
Pf. whitmori	Melioidosis	*aerogenes*	
Past. pestis	Plague	*Proteus sp.*	
boviseptica	Haemorrhagic septicaemia of cattle	*N. gonorrhoeae*	Gonorrhoea

Other antibiotics or sulphonamide compounds are known to inhibit some of the above bacteria but clinical results in treating their corresponding diseases have varied considerably in relation to three main factors, these being the ability to maintain a sufficiently high concentration of the therapeutic substance in the blood, the toxicity of the substance, and the liability to development of resistant bacterial strains Chloromycetin has advantages as regards high concentration in the blood and low toxicity and, so far, no evidence has been obtained regarding the evolution of chloromycetin-resistant strains Such advantages may possibly become apparent during clinical trials with chloromycetin on the various diseases represented in the above table

With such clinical trials in view, some notes, believed to have both general and local interest, are given as follows

Enteric Fevers and other Salmonella Infections —Twenty Malayan strains of *Salm typhi* obtained by blood culture from patients were found equally susceptible to chloromycetin by *in vitro* tests Among typhoid cases treated in Malaya by WOODWARD *et al* (1948), also later, relapses occurred in three, but the recurrent symptoms responded to a second course of chloromycetin —without increase of dosage In these three cases, the strain of *S typhi* isolated from the blood on relapse was compared with that originally cultured Using *in vitro* tests, no evidence was found that the organisms isolated on relapse had become chloromycetin-resistant

Apart from the typhoid group of bacteria, a variety of *Salmonella* organisms occur in Malaya (GREEN and MANKIKAR, 1939), and these include the species *senftenberg*, *bovis morbificans*, *enteritidis*, *cholerae-suis*, *london* and *typhi murium* —one or other being responsible for obscure fevers of varying duration, "food poisoning," joint infections and meningitis in infants A point of significance is that the ready inhibition *in vitro* of Malayan salmonella organisms by chloromycetin (also the known beneficial effects in typhoid fever) gives a clear indication for specific treatment of this important group of disease In the Federation of Malaya during 1947 there were 839 known cases of enteric infection with 178 deaths (MACGREGOR, 1947)

Cholera —Among the various bacteria tested against chloromycetin the cholera vibrios (Inaba and Ogawa strains) showed the widest zones of inhibition Chloromycetin, however, is believed normally to be absorbed rapidly from the bowel and its effect, in clinical cases, on large numbers of cholera vibrios swarming in the small intestine remains to be determined by trial in cases of the disease

Melioidosis —As reported by GREEN and MANKIKAR, 1949, *Pf whitmori* is susceptible to the effects of chloromycetin in clinically obtainable concentrations, but not to penicillin, streptomycin or polymyxin Later, it was found that a newly isolated Malayan strain of *Pf whitmori*, also five strains from Indo-China, were chloromycetin-susceptible in addition to the above and it

is inferred that chloromycetin would be effective in treating melioidosis, either alone or in combination with sulphonamides.

Pyelitis.—Pyelitis and cystitis are relatively common in the Federation of Malaya and are responsible for much chronic ill-health. In 1 year over 2,000 cases attended hospitals and clinics (MACGREGOR, 1946). Cases investigated bacteriologically at this laboratory range up to 300 or more yearly. Coliform organisms are the main infectors and 23 varieties have been identified in this laboratory, the commonest being *B. vesiculosus*, *B. coli commune*, *B. aerogenes*," *B. alkaligenes*, *B. coscoroba*, *B. columbense*, *B. coli communior*, *B. neapolitanum* and *B. khartoumense*—in order of frequency. Apart from these about one-quarter of the pyelitis cases are found infected with members of the *B. coli* group as yet unclassified, but probably of Asiatic origin.

Pyelitis due to members of the Proteus group, including *P. morgani*, is relatively uncommon, this group together with *Strep. faecalis* comprising only about 3 per cent. of infections. Such organisms were found susceptible to chloromycetin.

In another 3 per cent. of cases, however, the infecting organism is *Ps. pyocyanea*, Malayan strains of which are not affected by chloromycetin in concentrations up to 80 μg. per ml.*

In summary, the observed *in vitro* effects of chloromycetin (40 μg. per ml.) on local strains of "*B. coli*" and "*B. aerogenes*," *Proteus* sp. and *S. faecalis* which comprise over 90 per cent. of infections in pyelitis, together with the likelihood that chloromycetin would reach in the urine a concentration of 400 μg. per ml. or more, would justify the clinical trial of chloromycetin in this relatively common infection.

The Dysenteries.—Following the advent of sulphaguanidine for bacillary dysenteries, the specimens from such cases sent to this laboratory have shown a decrease. Hospital statistics show for 1947, however, admissions for "dysenteries and diarrhoea" as 6,358 with 645 deaths (MACGREGOR, 1947). Dysenteries have their main sites of infection in the large bowel, but it is believed that the major part of chloromycetin is absorbed from the small intestine; nevertheless, the gut tissues should contain fairly high concentrations, which would oppose further bacterial invasion, and it thus remains to determine clinically the efficacy of chloromycetin in bacillary dysenteries, also the necessity for its alternative use, particularly as sulphonamide-resistant strains of dysentery bacilli may occur (GALTON *et al.*, 1948).

As regards amoebic dysentery, it is noted that SMITH *et al.* (1948) found no significant decrease in numbers of motile *Entamoeba histolytica* after 48 hours at 37° C. when subject to 1 μg. per ml. of chloromycetin.

* *Ps. pyocyanea* is said to be susceptible to polymyxin D (SCHOENBACH *et al.*, 1948) also to polymyxin A, as well as streptomycin (BROWNLEE and BUSHBY, 1948). Malayan strains, however, are not inhibited by 10 μg. of streptomycin.

Diphtheria.—MacGregor (1946, 1947) reports the incidence in the Federation of Malaya of 645 cases of diphtheria within 2 years, of which patients 198 died—a death-rate of 30 per cent. Diphtheria patients, however, are often brought to hospital in late and irremediable stages of the disease.

Clinical trials with chloromycetin as an adjunct to diphtheria antitoxin may possibly show that chloromycetin has certain advantages over penicillin which is so often used as a supplementary antibiotic treatment.

Pneumococcal Infection.—During 1946, lobar pneumonia was responsible, in the Federation of Malaya, for 4,640 admissions to hospitals with 867 deaths. Cases of empyema for the same year were 286 with 49 deaths (MacGregor, 1946). On culture the pneumococcus is the organism most frequently isolated from empyema cases in Malaya. The pneumococcus is also responsible for about 36 per cent. of the local meningeal infections (see later) and such cases of pneumococcal meningitis mainly occur among Asian adults. Occasionally the pneumococcus is found in pure culture from pus withdrawn from deeply situated infections, notably thyroid abscesses. The types of pneumococcus encountered by us in Malaya include I, II, III, IV, VI, VII, IX, X, XII and XIII.

Meningitis.—The effective treatment of bacterial meningitis with an antibiotic substance requires ideally that the substance should not only (i) inhibit the infecting bacterium but (ii) be free from the likelihood of evolving resistant strains. Further, the antibiotic should (iii) be capable of diffusing from the blood through the choroid plexus in sufficiently high anti-bacterial concentrations—thus obviating the technical difficulties and infective risks of repeated intrathecal injections; also (iv) the antibiotic should be free from inherent toxicity, with the further risks of irreparable damage to neuronal tissues.

Streptomycin, although at present the only proved specific antibiotic for tuberculous meningitis, falls short of this ideal as regards the desiderata (ii), (iii) and (iv) above. Chorfmis *et al.* (1948) treated 63 subjects of tuberculous meningitis with streptomycin of whom 34 died and 29 survived. Of 21 patients discharged as "cured" four showed evidence of severe neuronal damage, and four had less severe "toxic" symptoms which tended to improve. The investigators, however, state that these "undesirable effects seem to be more the result of the disease in advanced stages than of the streptomycin in the doses we have come to use," * and they also state that "Intra-thecal streptomycin is essential in tuberculous meningitis."

As regards the treatment with streptomycin of meningitis due to *H. influenzae*, Smythe (1948) who had three deaths among 11 treated patients, recommended that all cases be treated with combined streptomycin and sulphonamides. Among his eight surviving patients there were three relapses.

* It is possible that the treatment of tuberculous meningitis by streptomycin may result in a large proportion of severe [illegible] certain forms of residual tubercular damage [illegible] would otherwise have been erased by death.

and among the three deaths there was one case in which the infecting strain of *H. influenzae* rapidly became streptomycin-resistant despite big doses.

As regards other antibiotics which may be used for treating meningitis, penicillin lacks a specific effect both on *H. influenzae* and *Myco. tuberculosis* and although it is known to inhibit *S. pneumoniae*, *N. meningitidis* and *Str. pyogenes*, it fails usually to pass the "blood-brain barrier" in sufficiently high concentrations when given intramuscularly and must be injected intrathecally (KOLMER, 1947). With regard to polymyxin D SCHOENBACH *et al.* (1948), when treating a case of purulent meningitis by intramuscular injection found none of the antibiotic in the spinal fluid. Again aureomycin, a new antibiotic obtained from a *Streptomyces* and active against gram-positive and gram-negative bacteria (FINLAND *et al.*, 1948) is said not to penetrate the blood-brain barrier (Annotation, *Lancet*, 1948).

Thus, in summary, the imperfections of streptomycin and the limitations of penicillin in treating bacterial meningitis, spur the investigator further to seek an antibiotic which may be more effective, less toxic than streptomycin, and more readily administered. That chloromycetin can be *given by mouth*, attain a concentration of 20 to 80 μg. per ml. in the blood, also attain, according to SMADEL *et al.* (1949), about half this concentration in the cerebrospinal fluid,† clearly points the way to a thorough clinical investigation of the value of chloromycetin in the treatment of meningitis. Preliminary *in vitro* tests regarding the specific effects of chloromycetin on the various causative bacteria are necessary. With this clinical investigation in view, the writers present a few relevant findings on meningitis in Malaya.

MACGREGOR (1946) reports that 252 cases of meningitis were treated in hospitals of the Malayan Federation during that year. The general death rate was 71 per cent. Of the 24 meningococcal patients, 33 per cent. died, and among 56 cases (listed as "Tuberculosis of the Central Nervous System") the death rate was 86 per cent.

Among 162 Malayan cases of meningitis bacteriologically investigated by the writers, the infecting bacteria occurred in the following percentages: *Str. pneumoniae* (36 per cent.), *N. meningitidis* (2_ per cent.), *H. influenzae* (16 per cent.), *Str. pyogenes* (12 per cent.), *Myco. tuberculosis* (11 per cent.), *Str. viridans* (2 per cent.), and *Salmonella* sp. (1 per cent.).

Of the above bacteria, *Str. pneumoniae*, *N. meningitidis* and *Str. pyogenes* are inhibited by penicillin and sulphonamides.

The remaining four bacteria, *H. influenzae*, *Myco. tuberculosis*, *Str. viridans* and *Salmonella* sp., which are together responsible for 30 per cent. of the meningeal infections in Malaya, require for their treatment (in accordance with the foregoing) more effective and more conveniently administered antibiotics than

* Penicillin-sensitive strains of *H. influenzae* has so far not been encountered by the writers in Malaya.

† No toxic effects on the cerebral tissues with this dosage has so far been reported.

Gonorrhoea.—Gonorrhoea is prevalent in Malaya, a proportion of cases nowadays being "self treated" with sulphonamides. MacGregor (1946) reported that over 14 000 cases attended hospitals and clinics for treatment.

The organism *Neisseria gonorrhoeae* is, of course known to be inhibited by practicable concentrations of sulphonamides and penicillin, while streptomycin has been successfully used in treatment by Chinn *et al.* (1947) with only slight toxic manifestations.

The writers found that two freshly isolated Malayan strains of *N gonorrhoeae* were readily inhibited by 5 to 10 μg. of chloromycetin and, as regards the clinical application of this finding, it is believed that, during the administration of chloromycetin by mouth, the sub-urethral tissues may be subject to concentrations of 40 to 80 μg of chloromycetin per ml. the surface epithelium being subject to concentrations about 10 times greater

In certain cases, therefore, where the possibility of encountering sulphonamide or penicillin resistant strains exists, or where it is wished to avoid intramuscular injections or the possible toxic effects of sulphonamides chloromycetin may afford a useful alternative treatment for gonorrhoea.

FURTHER CLINICAL TRIALS

Further investigations on the known response of typhoid and scrub-typhus to chloromycetin have already been undertaken in Malaya by Smadel and colleagues but as regards the use of this new antibiotic in other diseases, careful consideration will, of course be necessary in order to obtain sound information, to conform with the best interests of the patients, and to avoid wastage. The *in vitro* tests mentioned in this paper were carried out to provide some preliminary guidance in these respects.

Thus, further clinical trials in a variety of diseases need to be undertaken on a carefully controlled basis—primarily among infections caused by bacteria which are known to be susceptible to practicable concentrations of chloromycetin and a brief classification to be used serially for selecting cases is suggested as follows (i) No other specific treatment is available (ii) Specific treatment is available but clinical results are not entirely satisfactory chloromycetin possibly being a more effective alternative (iii) specific treatment is available and satisfactory but chloromycetin is more convenient to administer

SUMMARY

In vitro tests show that a series of Malayan pathogenic bacteria are affected by chloromycetin when subjected *in vitro* to such concentrations of the antibiotic as are obtainable during administration by mouth.

Suggestions are made as regards the selection of cases for clinical trials—to be conducted primarily on diseases, the causative bacteria of which have been shown susceptible to practicable concentrations of chloromycetin and, in general, it is considered that the therapeutic uses of chloromycetin are best

determined by investigations designed to shed the fullest light not only on its efficacy but also on the necessity for its use and convenience of administration —in relation to other available therapy

REFERENCES

ANNOTATION (1948) *Lancet*, 2, 618
BROWNLEE, G, & BUSHBY, S R M (1948) *Ibid*, 1, 127
CHINN, B D, PUTNAM, L E, TAGGART, S R, & HERWICK, R P (1947) *Amer J Syph*, 31, 268
CHOREMIS, K, ZERVOS, N, CONSTANTANIDES, V, & PANTAZIS, S (1948) *Lancet*, 2, 485
COLLIER, H O J (1948) *Research*, 1, 501
EHRLICH, J, BARTZ, Q R, SMITH, R M, JOSLYN, D A, & BURKHOLDER, P R (1947) *Science*, 106, 417
FINLAND, M, COLLINS, H S, & PAINE, T F, Jr (1948) *J Amer med Ass*, 138, 946
GALTON, M M, MITCHELL, R B, CLARK, G, & RIESEN, A H (1948) *J infect Dis*, 83, 147
GREEN, R, & MANKIKAR, D S (1939) *Ann Rep Inst Med Res, Kuala Lumpur*, 28
——— & ——— (1940) *Ibid*, 34
——— & ——— (1949) *Brit Med J*, 1, 308
KOLMER, J A (1947) *Penicillin Therapy* New York D Appelton-Century Co, pp 83, 89, 283
LEY, H L, Jr, SMADEL, J E, & CROCKER, T T (1948) *Proc Soc exp Biol Med*, 68, 9
MACGREGOR, R B (1946) *Rep Med Dept Malayan Union*, pp 26, 42, 60, 64, 65
——— (1947) *Ibid*, pp 10, 11
SCHOENBACH, E B, BRYER, M S, BLISS, E A, & LONG, P H (1948) *J Amer med Ass*, 136, 1096
SMADEL, J E, & JACKSON, E B (1947) *Science*, 106, 417
——— & ——— (1948) *Proc Soc exp Biol Med*, 67, 478
———, WOODWARD, T E, LEY, H L, Jr, PHILIP, C B, TRAUB, R, LEWTHWAITE, R, & SAVOOR, S R (1948) *Science*, 108, 160
———, ———, ——— & LEWTHWAITE, R (1949) *Chloromycetin in the treatment of Tsutsugamushi Disease (Scrub Typhus)* In preparation
SMITH, R M, JOSLYN, D A, GRUHZIT, O M, MCLEAN, I W, PENNER, M A, & EHRLICH, J (1948) *J Bact*, 55, 425
SMYTHE, P M (1948) *Lancet*, 2, 485
SWIFT, P N (1948) *Ibid*, 1, 133
WOODWARD, T E, SMADEL, J E, LEY, H L, Jr, GREEN, R, & MANKIKAR, D S (1948) *Ann int Med*, 29, 131

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HAEMOGLOBIN ESTIMATION BY THE CYAN HAEMATIN METHOD

MODIFICATION FOR USE IN WARM CLIMATES*

BY

H LEHMANN, M D (BASLE), PH D (CANTAB), F.R I C ,

AND

R B BAIRD, M B , CH B (EDINBURGH),

From the Department of Physiology, Makerere College and Mulago Hospital, Kampala, Uganda

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On standardizing Sahli haemoglobinometers by the cyan hematin method of KING and GILCHRIST (1947), we were surprised to find the Sahli method to give persistently low values whereas it usually errs in the opposite direction, due to the fading of the permanent colour standards Furthermore, on using the cyan haematin method on African and European patients we found that our results were about 5 per cent. higher than those obtained by the alkaline haematin method of CLEGG and KING (1942) That the former results were erroneous became clear when the bloods of well-nourished Africans were investigated Working at an altitude of 3,800 feet, we found the mean corpuscular haemoglobin concentration near the maximum of 36 per cent ,† when the alkaline haematin method was used, but the cyan haematin method gave us values well above this theoretically possible figure (Table I)

Working with bloods with a high haemoglobin concentration, we could now see with the naked eye that a fine turbidity appeared when, in using the cyan haematin method, cyanide was added to the acid haematin That this

* Work carried out under the scientific direction of the Colonial Medical Research Committee in a scheme financed from the Colonial Welfare and Development allocation

We are grateful to the Director of the Colonial Medical Research Service and the Director of Medical Services, Uganda for permission to publish this paper

† In assuming 36 per cent. as the maximum mean corpuscular haemoglobin concentration we follow the opinion of the majority of workers It is noteworthy that WINTROBE, who is still quoted by WHITBY and BRITTON (1946) as belonging to the minority who consider 38 per cent as the upper limit, now also adheres to 36 per cent as the maximum (WINTROBE, 1946)

TABLE I.

BLOOD HAEMOGLOBIN CONCENTRATION IN 14 WELL NOURISHED EAST AFRICANS.
(HAEMOGLOBIN ESTIMATED BY ALKALINE AND CYAN HAEMATIN METHODS, MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATIONS CALCULATED FOR BOTH.)
CYAN HAEMATIN METHOD PERFORMED AT 18° TO 24°C

Haemoglobin (grammes per cent.).		Mean corpuscular haemoglobin concentration (per cent.).	
Alkaline haematin method.	Cyan haematin method.	Alkaline haematin method.	Cyan haematin method.
13·8	12·5	33·3	34·7
13·9	14·0	32·7	33·9
14·6	14·8	32·9	34·9
14·0	14·8	31·3	36·2
15·2	16·05	34·6	36·1
15·4	17·25	33·4	37·4
15·8	16·2	35·8	37·7
15·9	16·75	34·5	37·1
16·0	17·1	34·6	37·2
16·1	17·1	34·6	38·9
16·2	17·5	35·9	39·9
16·9	17·15	36·1	38·6
17·7	18·65	36·1	38·1
17·8	19·0	35·9	38·4

turbidity was present also when bloods with lower haemoglobin concentration were investigated could be gathered from the fact that in these samples on filtering through a Whatman No. 42 filter paper the extinction of light (King's photo-electric colorimeter with an Ilford Tricolour Green filter 404 was used), fell by about 8 per cent., whereas a cyan haematin solution prepared from haematin lost only 3 to 4 per cent. of its density on filtering. No loss of density was observed when cyan haematin solutions prepared from haematin were centrifuged at 3,000 r.p.m. for 15 minutes. The same treatment led, however to a loss in density in cyan haematin solutions prepared from blood. The results were variable and although there was always a loss in density on centrifuging, there were differences of 100 per cent. in the density loss in duplicate samples.

A precipitate could be obtained from the bottom of the centrifuge tubes, and it consisted of protein and other soluble matter. Furthermore, the precipitate was obtained equally when untreated, oxalated and heparinized bloods or washed blood cells were used, but was not seen when pure haemoglobin was employed (Tables II and III). It seemed, therefore, that the non-haemoglobin constituents of the red cells were responsible for the turbidity

TABLE II

HAEMOGLOBIN CONTENT OF WASHED RED BLOOD CORPUSCLES.

(OXALATED HUMAN BLOOD CENTRIFUGED, PLASMA REMOVED, CELLS WASHED THREE TIMES WITH SALINE BY CENTRIFUGING, SALINE REMOVED, WATER ADDED TO ORIGINAL VOLUME.)

Sample	Method	Incubation with HCl: Temperature in °C	Incubation with HCl: Time in mins	Incubation with HCl: Concentration of HCl in N	Extinction of light compared with water King's photo-electric colorimeter, 1 cm. cell, Ilford Tricolour Green Filter 404 ($E \times 100$)	Haemoglobin (grammes per cent.) calculated from extinction
A	Alkaline haematin	—	—	—	—	19.65
	Cyan haematin	20	15	0.1	53	20.7
	"	29	15	0.033	50	19.5
B	Alkaline haematin	—	—	—	—	7.70
	Cyan haematin	26	15	0.1	22	8.6
	"	26	15	0.033	20	7.8

TABLE III.

DENSITY OF CYAN HAEMATIN SOLUTION FROM PURE HAEMOGLOBIN.
(HAEMOGLOBIN GROUND IN WATER, FILTERED THROUGH NO. 1 WHATMAN FILTER PAPER, 5 ML. FILTRATE + 10 ML. HCl, 4 ML. 5 PER CENT. NaCN ADDED, WATER ADDED TO 20 ML.)

Sample.	Incubation in HCl for 15 mins. at 30° C. Concentration of HCl in *N*	Extinction of light as in Table II. ($E \times 100$.)
A	0·1	14
A	0·033	14
B	0·1	22
B	0·033	22
C	0·1	27
C	0·033	27
D	0·1	58·5
D	0·033	59

There was a difference in the amount of turbidity seen when European and African bloods were compared European bloods showed no or very little turbidity when incubated with HCl immediately after thay had been obtained from the subjects Left standing, however, at room temperature for a few hours, and then used for haemoglobin estimation by the cyan haematin method, they showed the turbidity without exception—though still to a lesser extent than the blood samples from Africans

KING and GILCHRIST (1947) have pointed out the advantages of the cyan haematin over the alkaline haematin method To these advantages has to be added the stability of the cyan haematin standard solution as compared with the instability of the alkaline haematin standard solution often experienced in tropical climates We have now been able to overcome this particular difficulty to some extent by storing in the refrigerator alkaline haematin solutions with a density corresponding to 30 gramme per cent haemoglobin and diluting them before use One such standard solution remained constant in extinction values for over 3 months The same solution three times diluted and stored under the same conditions lost 37 per cent. of its density However, the disadvantage of this procedure is the need to bring the standard solution to room temperature before use. Cyan haematin standard solutions corresponding to 14 8 gramme per cent haemoglobin and prepared both from ox blood haematin with an Fe percentage of 8 41 (prepared after DELORY, 1943) and from commercial haematins with an Fe percentage of 8 25 and 8 29, respectively, were stored at room temperature in a transparent bottle in the light and in a dark bottle in a closed cupboard In 10 weeks in which the temperature at noon rose gradually from 18° to 35° C the density loss in all three samples under both conditions was only 0 25 per cent per week, the density loss over the following 10 weeks was even lower We were therefore anxious to find a way to use the cyan haematin method while avoiding the turbidities described above

The following four procedures produced solutions where no turbidity could be seen with the naked eye or demonstrated by density loss after centrifuging The results agreed closely with those obtained by the alkaline haematin method

(*a*) Incubation of the blood in 0 1 N HCl at + 4° C
(*b*) , „ 0 1 N HCl + 0 033 M NaCl
(*c*) , , 0 033 N HCl instead of in 0 1 N HCl
(*d*) , , 0 1 N or 0 033 N NaOH

Although we have not definitely excluded that the non-haemoglobin constituents of the red cells were responsible for the turbidity by themselves (Tables II and III), the above procedures were tried out under the assumption that in acid solution and at raised room temperature the globin underwent changes when the non-haemoglobin constituents of the cells were present These changes, we assumed, made the globin liable to be precipitated by cyanide at an alkaline pH Globin—a histon—can be expected to undergo partial denaturation when other large molecules are present in the solution

TABLE IV

HAEMOGLOBIN ESTIMATION IN OXALATED HUMAN BLOOD.

Method.	Temperature in °C.	Incubation with HCl. Time in mins.	Incubation with HCl. Concentration of HCl in N	NaCl in M.	Extinction of light in per cent. as in Table II.	Haemoglobin (grammes per cent.) calculated from extinction.	Per cent. difference from alkaline haematin value.
Alkaline haematin	—	—	—	—	—	17.1	—
Cyan haematin	37	15	0.1	—	51	19.9	+16
	37	120	0.1	—	51	19.9	+16
	4	15	0.1	—	43.5	17.0	— 0.6
	4	120	0.1	—	46.5	18.1	+ 6
	37	15	0.1	0.033	43.5	17.0	— 0.6
	37	120	0.1	0.033	51	19.9	+16
	37	15	0.033	—	43.5	17.0	— 0.6
	37	120	0.033	—	45	17.5	+ 2

TABLE V

OXALATED HUMAN BLOOD. HAEMOGLOBIN CONTENT 11.0 GRAMMES PER CENT (ALKALINE HAEMATIN METHOD).

HAEMOGLOBIN ESTIMATION BY CYAN HAEMATIN METHOD. EFFECT OF COOLING THE ACID HAEMATIN BEFORE ADDITION OF CYANIDE.

Incubation in 0.1 N HCl. First 30 mins. at.	Incubation in 0.1 N HCl. Followed by 5 mins.	Extinction of light as in Table II (E 100)	Haemoglobin (grammes per cent.) calculated from extinction.	Per cent. difference from alkaline haematin value
0° C.	0° C.	28.5	11.1	+ 1
0° C.	37° C.	29.9	11.65	+ 6
37° C.	37° C.	33	12.85	+17
37° C.	0° C.	30.5	11.9	+ 8

TABLE VI

OXALATED HUMAN BLOOD HAEMOGLOBIN CONTENT 14 8 GRAMMES PER CENT (ALKALINE HAEMATIN METHOD)

HAEMATIN ESTIMATION BY CYAN HAEMATIN METHOD

Incubated for 15 mins at 37° C before addition of cyanide	10 mins after addition of cyanide			30 mins after addition of cyanide		
	Extinction of light as in Table II ($E \times 100$)	Haemoglobin (grammes per cent) calculated from extinction	Per cent difference from alkaline haematin value	Extinction of light as in Table II ($E \times 100$)	Haemoglobin (grammes per cent.) calculated from extinction	Per cent difference from alkaline haematin value
0 1 N NaOH	35 3	13 8	—7	37	14 4	—3
0 033 N NaOH	37 5	14 6	—1	37 8	14 7	—0 7
0 033 N HCl	38	14 8	±0	37 9	14 75	—0 3

(*a*) *Temperature* —The appearance of turbidity in the cyan haematin method depends to some extent on the temperature at which the acid haematin is formed from blood, 15 minutes' incubation in 0 1 N HCl at + 4° C before the addition of the sodium cyanide solution results in values equal to those obtained by the alkaline haematin method, whereas a temperature > + 25° C can be expected to raise the density by > 5 per cent However, at lower temperature the time factor becomes important and incubation at + 4° C in 0 1 N HCl for 120 minutes instead of 15 minutes results in sufficient turbidity to raise once again the density of the cyan haematin solution by > 5 per cent (Table IV)

The effect of raised temperature during incubation with 0 1 N HCl is, however, only partially responsible for the turbidity observed on adding the sodium cyanide Some of the turbidity can be avoided when the acid haematin solution is cooled before the cyanide is added (Table V)

(*b*) *Salt Effect* —Addition of 0 033 M NaCl to the 0 1 N HCl solution also prevents the appearance of turbidity (Table IV) and no temperature precaution has to be taken Yet the turbidity appears when the HCl incubation is prolonged beyond 15 minutes, and after 120 minutes is the same whether NaCl had been added or not (Table IV)

(*c*) *Concentration of HCl* —A good way to avoid the appearance of turbidity is to use 0 033 N HCl instead of 0 1 N HCl for the incubation of the blood This method is little dependent on the time factor, there being no difference between the values obtained by 15 or 30 minutes' incubation, and even 120 minutes' incubation in 0 033 N HCl will result in a turbidity raising the density by < 5 per cent of the true value (Table IV)

(*d*) *Incubation in Alkali instead of Acid.*—Very clear solutions can be obtained on the addition of cyanide to alkaline haematin solutions. The density of such cyan haematin solutions does not differ from those obtained when the same amount of blood was incubated in acid. The colour development is somewhat slower when 0·1 *N* NaOH is used than if 0·033 *N* NaOH is employed (Table VI.).

Summary

The cyan haematin method for the estimation of haemoglobin concentration in blood—performed at the high temperatures experienced in a tropical climate—yields higher results than the alkaline haematin method. This difference is greater the higher the temperature at which the blood is incubated with 0·1 *N* HCl to form acid haematin prior to its conversion into cyan haematin. It is suggested that this difference is due to a partial denaturation of the histon globin when incubated in 0·1 *N* HCl in the presence of the non haemoglobin constituents of the red cells—leading to a turbidity on the addition of cyanide.

This turbidity can be lessened by cooling the acid haematin solution before the cyanide is added to form cyan haematin. It can also be avoided by lowering the temperature at which the acid haematin is formed, or by the addition of salt, or by transforming the acid haematin into alkaline haematin before the addition of cyanide. The best procedures to use are incubation with either 0·033 *N* HCl or 0·033 *N* NaOH to form the haematin before its conversion into cyan haematin without any other alteration of the original method.

The results thus obtained correspond closely to those arrived at by the alkaline haematin method of Clegg and King (1942).

References

Clegg, J. W. & King, E. J. (1942). *Brit. Med. J.* 2, 329.
Delory, G. E. (1943). *Analyst*, 68, 5.
King, E. J. & Gilchrist, M. (1947). *Lancet*, 253, 201.
Whitby, E. H. & Britton, C. J. C. (1946). *Disorders of the Blood.* London: J. & A. Churchill.
Wintrobe, M. M. (1946). *Clinical Haematology.* London. Henry Kimpton.

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SOME PECULIAR CASES OF GANGRENE AND THEIR POSSIBLE RELATIONSHIP TO TROPICAL PHLEBITIS*

BY

MICHAEL GELFAND, M D, M R C P,
Physician, Salisbury Native Hospital, Southern Rhodesia

The purpose of this article is to describe three interesting cases of gangrene in the African, with an attempt to correlate the condition in these cases with tropical phlebitis or thrombophlebitis

Thrombosis of the larger veins with or without phlebitis is a well recognized complication of acute infections, such as pneumonia and typhoid fever, and after operations Much has been written recently on this subject, particularly on its prevention and treatment by heparin and dicourmarol A primary phlebitis was first described in Africa in 1941 by FISHER, of Northern Rhodesia He found the disease mostly in young African adult males, but occasionally also in the European Although the femoral vein is the most common one to be affected, yet other veins, especially the subclavian, jugular, mesenteric, portal and splenic may be attacked either singly or less frequently in combination The onset of the condition tends to be abrupt with a febrile disturbance of varying severity and pain and tenderness over the inflamed area, followed within a few hours by oedema of the affected extremity The oedema may be intense The course of the illness varies In some it may be mild and of a few days' duration but in others it may be more severe, with marked fever and even delirium lasting many days

FISHER and his colleagues (1947) claim to have shown that only a short portion of the vein is usually inflamed and by special staining methods inclusion bodies can be shown in the large epitheloid cells, which they refer to as polyblasts They suggest that the condition be referred to as tropical phlebitis rather than idiopathic thrombophlebitis

The oedema is interesting, as in cases of femoral thrombosis after a lumbo-sympathetic block is performed, it vanishes rapidly The oedema is thus due to an associated arteriolar spasm since it disappears when the influence of the sympathetic on the arteries and arterioles is eliminated GELFAND (1948) has published a series of cases of tropical phlebitis showing that lumbo-sympathetic block may be employed in the treatment of the more severe cases of femoral thrombosis with oedema. It is probable that in these cases, where arterial or arteriolar spasm is intense, gangrene may supervene since the blood supply may be completely cut off That this can occur is shown in Case 3, where

* I wish to thank Dr G R Ross, Acting Secretary for Health, Southern Rhodesia, for his kind permission to publish this paper

the child developed lobar pneumonia and a week later the left leg and foot became markedly swollen followed by gangrene of all the toes. Obviously in this case the phlebitis was secondary to the pneumonia. Nevertheless, it is recorded here as it affords an explanation for the occurrence of gangrene in cases of thrombophlebitis.

GELFAND (1947) published a series of African cases of symmetrical gangrene in the lower extremities, each with a more or less characteristic onset running a similar course. In every case pain and oedema occurred simultaneously in both feet, soon followed by gangrene of all the toes or of the entire feet. The striking feature in these cases was the symmetrical distribution—a feature not usually seen in tropical phlebitis. Normally whilst more than one vein may be affected, it is rare for the disease to be symmetrical. In a later communication CHARTERS and MANSON BAHR (1948) suggest that these cases of symmetrical gangrene with oedema may have a similar aetiology to that occurring in tropical phlebitis. This may apply perhaps even more to unilateral cases of gangrene of the fingers, toes and less frequently of a more extensive distribution.

In this communication two cases of gangrene, limited to the fingers of a hand and preceded by oedema, are described and presented as cases of tropical phlebitis with an arteriolar spasm or perhaps an arteritis with thrombosis. Should inflammatory changes similar to those found in the veins be shown in the arteries, the condition might preferably be referred to as tropical angeitis.

The case histories of the three patients are as follows

CASE 1 CHIKAONDA.—An adult male African, aged about 43 years, was admitted to the Salisbury African Hospital on 10th January 1946, complaining of blackness of the fingers of the left hand (Plate 1). The patient stated that he was perfectly fit until 2 weeks prior to the onset of the condition. He first felt pain in all the fingers, but not in the thumb. About 4 days later all the fingers became swollen. After another 4 days he noticed that the pads of the fingers had turned black and become cold. The blackness commenced in the third digit, then spread to the fourth, later to the second and finally to the fifth. The pads of his fingers felt dead.

There was no previous history of note The patient was employed by the Milling Company Salisbury where he transferred grain from the trucks to the main store From 1928 to 1932 he worked underground in one of the gold mines of Southern Rhodesia. His diet consisted of rice mealie meal (maize) porridge and meat twice daily but vegetables rarely He was accustomed to smoking three cigarettes day for the past 3 years.

On examination the patient looked perfectly fit. The conjunctivae were well coloured and the gums healthy The skin showed no signs of pellagra, itamin A deficiency or leprosy The lymphatic glands were not enlarged.

The heart was normal in size and shape the blood pressure being 140 90 The rine was free of albumin, sugar and casts. His lungs were clear (confirmed radiologically). An X-ray of the neck showed no evidence of cervical rib.

On abdominal examination the liver edge was palpable and slightly hard. The central nervous system was normal. His blood Wassermann reaction was negativ The tools were free of parasitic ova.

On examination of the left upper extremity there was gangrene of the first four fingers affecting especially the soft tissues and being most marked in the little finger It then gradually diminished towards the thumb which only had small bleb at the tip of its volar aspect. The gangrene had attacked only the volar portions of the fingers, the gangrenous areas being of blackish colour These parts were cold and without sensation. The fingers above the gangrenous portions were of normal temperature and with no sensory

PLATE 1.—Note the distribution of the gangrene in the digits of the adult African male with maximal involvement in the little finger

PLATE 2.—In this plate is seen the distribution of the gangrene in the right hand of the young female patient, the most severe damage being present in the index finger. The slight changes in the fourth and fifth digits have already cleared

FIG. 3. The gangrene of the toes with the oedema of the foot are clearly shown

loss The right radial artery could not be palpated, but the right brachial artery was felt pulsating as vigorously as the brachial artery on the left side

CASE 2 TIAPO—An African female, aged about 15 years, was admitted to the Salisbury African Hospital on 19th of December, 1945, from a native reserve near Enkeldoorn, about 100 miles south of Salisbury The history was that of a sore hand of 7 weeks' duration The patient stated she was perfectly fit until 7 weeks prior to admission when all the fingers of the right hand became swollen and painful She mentioned that the swelling was the first sign to appear, followed shortly after by pain The pain was fairly severe and, as a result, she was unable to move the fingers A week after the onset of the condition, the first digit became cold and black at its tip Soon afterwards the other fingers followed suit The pain lasted about 1 month

The patient's diet prior to admission consisted of "sadza" (maize), sour milk and raw roots, vegetables from the veld, kafir oranges and beer daily

On examination she appeared to be well covered and healthy There were no obvious signs of anaemia and the blood examination showed 80 per cent haemoglobin The heart and lungs were normal, the blood pressure being 95/60 An X-ray of the cervical region revealed no evidence of an additional rib On abdominal examination there was no enlargement of the liver or spleen The Wassermann reaction of the blood was negative The leucocyte count was normal except for a 10 per cent eosinophilia The urine was free of sugar, albumin and ova and the stools were normal on microscopical examination

On inspection of the right hand, there was gangrene of the thumb up to the middle of the proximal phalanx, the index finger extending to the proximal inter-phalangeal joint, the middle one almost up to the terminal interphalangeal joint, but only the tip of the ring digit (measuring about ¼ inch in diameter) and a very small portion of the little finger (Plate 2) It will be seen that the gangrenous changes diminished from the thumb towards the little finger The maximal damage affected the pulps or volar aspects of the fingers The gangrene was most intense in the thumb, index and middle digit, and was blackish in colour with greyish areas due to pockets of pus The nails of the thumb, index and middle fingers were lustreless and of a blackish-brown hue, but those of the fourth and little finger were apparently unaffected The diseased portions of the fingers were cold

The small areas of the fourth and fifth digits healed after about 2 weeks, the dead skin peeling and being replaced by healthy tissue On the whole of the right hand the integument was blacker and dryer than that on the corresponding surfaces of the left This dry skin later cracked, peeled and after a few weeks was replaced almost entirely by healthy skin The radial artery could not be felt in the right forearm, but the brachial pulsation was present in the arm and was of the same intensity as that palpated in the left brachial artery

CASE 3—The patient was an African female aged about 2¼ years, brought into hospital by her mother, who stated that the child was well until a week prior to admission when she developed a cough and fever, which were still present The day before admission the left leg and foot began to swell and were painful Soon after, blisters appeared on the foot and she observed that the left big toe had become black The others followed suit within the next 2 days

On admission the child looked ill and was dyspnoeic, the respiration being of a grunting character Numerous crepitations were heard over the whole of the left lung An X-ray of the chest showed a pneumonic consolidation of the left lung The temperature was 102° F and the white cell count was 16,400 with 60 per cent neutrophiles, 39 per cent lymphocytes and 1 per cent monocytes A slight anaemia was present, the total red blood corpuscles being 3½ million per c mm and the haemoglobin 77 per cent There were no malarial parasites in the blood The mother's Wassermann reaction was negative

The whole of the left foot and leg, as far as the upper third, was markedly swollen and oedematous Several blisters filled with sero-sanguineous fluid were seen over the dorsum and plantar aspects of the foot and on the lower third of the leg The left leg, 2 inches above the ankle, measured 5¼ inches in circumference as compared with the corresponding portion of the right leg which was 4 inches The left foot was hotter than the right one The left popliteal artery could be felt pulsating, but not the arteries in the foot This may have been due to the gross oedema present, since when the swelling subsided, the dorsalis pedis was palpable On X-ray examination of the leg and foot no abnormalities of the bones were seen

The striking feature of the case was the gangrene affecting all the toes, but most marked in the big toe and successively less towards the little one. (Plate 3.)

The child was given penicillin and sulphonamide therapy and her general condition soon improved. The oedema of the leg and foot diminished fairly rapidly during the next few days. The blisters ruptured, leaving behind superficial ulcers of the skin. The gangrenous areas on the toes gradually separated off at the lines of demarcation, the maximum damage being in the great toe and the least in the little one.

The diagnosis in this case was of lobar pneumonia with thrombophlebitis of the femoral vein causing the marked oedema of the foot and ankle. The gangrene is best explained as being due to spasm occurring in the arteries or arterioles of the foot, as result of the thrombophlebitis.

Commentary

The ordinary causes of gangrene, such as hypertension and arteriosclerosis, diabetes, thromboangiitis obliterans and Raynaud's disease can readily be excluded in the differential diagnosis of these three cases. Similarly embolism from the heart or massive thrombus of the left auricle can be dismissed. In none of these cases could ergot poisoning be traced. The interesting features in these cases and in those the author described in 1947 were the swelling and the gangrene limited to the digits or tips of the digits or the toes. Further the oedema was always associated with or preceded by the gangrene. In other words, a pure arterial lesion could not explain the oedema, which can best be accounted for by a thrombophlebitis with an associated arterial or arteriolar spasm. I have shown earlier in this paper that in tropical phlebitis the oedema disappears after a lumbo-sympathetic block.

Whereas in the majority of cases of tropical phlebitis the arteriolar spasm is not sufficient to lead to gangrene, yet occasionally this may follow Case 3 is not presented as a case of tropical phlebitis with gangrene, as an acute infective process in the lungs was responsible for the femoral thrombosis, but the case is utilized to demonstrate that in thrombophlebitis with oedema, gangrene of the digits may set in from the associated arteriolar spasm.

Summary

1 Three peculiar cases of gangrene of the fingers or toes in Africans are described. In one case pneumonia preceded the gangrene but in the remaining two no apparent illness was present. Oedema preceded the gangrene in each case.

2. It is suggested, in view of the fact that the oedema in tropical phlebitis disappears rapidly after a lumbo-sympathetic block that such peculiar cases of gangrene seen in the African may be tropical phlebitis in which there is also an associated arterial spasm or arteritis with thrombosis.

3. Its suggested relationship to tropical phlebitis is discussed.

REFERENCES.

Charters, A. D & Manson-Bahr, P. E. C. (1948). (Correspondence.) *Brit. med. J* 1 520.

Fisher, A. C., Fisher, M. M. & Lendrum, A. C. (1947). *J Path. Bact.*, 49 405

Gelfand, M. (1947). *Brit med. J* 1 847

——— (1945). *S Afr med J* 19, 447

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A CASE OF CUTANEOUS AMOEBIASIS

BY

WILLIAM ARMSTRONG, M B , B S (MELB),
Mission Hospital, Lenakel, Tanna, New Hebrides
(Communicated by Professor P A BUXTON, C M G , F R.S)

In the course of some years' medical practice in the New Hebrides I have seen two patients suffering from cutaneous lesions which appeared to be due to amoebiasis (*Entamoeba histolytica*, presumably) An illustration of one of these appears overleaf

This patient came into hospital in April, 1947, with a fungating mass over his sacrum The first impression was of a fungating sarcoma The growth was coarsely papillomatous with copious offensive discharge filling the interstices of the growth The history given was that it started nearer the anus about 3 years before when he was working on another island as part of a labour force recruited by the American military forces He had received no treatment (probably had not reported sick) At the time I first saw him the area between the lesion and the anus was clear of papillomata but showed evidence of scarring The discharge was rich in amoebae

The patient was immediately put on a course of emetine grain 1 per diem and the local lesion dressed with a 1 per cent solution of carbarsone Discharge ceased within 3 or 4 days and the carbarsone dressings were replaced by a paint of salicylic acid in tinct benz co Emetine was continued for 10 days

During the rest of his stay in hospital the area remained dry but the salicylic acid paint was very slowly if at all, effective in reducing the area of the warty mass. Recourse was then had to glacial acetic acid, which rapidly cleared the area.

Latest information, in May 1948, is that there has been no recurrence. The photograph was taken on the second or third day of treatment.

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CLINICAL AND BIOCHEMICAL STUDIES IN CHOLERA AND THE RATIONALE OF TREATMENT

BY

M H GHANEM, M D,

AND

M N MIKHAIL, M PH, D B A*

(Medical Unit, Faculty of Medicine, Farouk I University, Egypt)

More knowledge about the pathological physiology of cholera and the various mechanisms underlying the fatal outcome in this disease, will certainly improve its prognosis With this aim in mind, a number of cholera cases has been studied clinically before, during and after various lines of treatment, the rationale of these is discussed The severity of the condition was measured clinically and with the aid of laboratory investigations The various complications met with are recorded

I THE CLINICAL STUDY includes General condition of the patient on admission graded according to severity, Frequency and duration of vomiting and diarrhoea, Dehydration as shown by skin, eyes, tongue, thirst, veins, abdomen and urine volume, Temperature, Circulatory system (pulse and blood pressure), Nervous system (twitches, reflexes and paresis), Other findings in the skin and limbs, Complications and sequelae

II BIOCHEMICAL ANALYSIS includes —(a) *Blood* Specific gravity of blood (method of Phillips and Van Slyke), Specific gravity of plasma (method of Phillips and Van Slyke), Haemoglobin (Sahli's method), Haematocrit (method of Phillips and Van Slyke), Amount and percentage of plasma proteins (method of Phillips and Van Slyke), Urea (nesslerization), Sugar (method of Folin-Wu), Potassium (method of Kramer and Tisdal), Chlorides (direct titration), While taking blood specimens for analysis, its viscosity was noticed (b) *Urine* Amount (24 hours), specific gravity, reaction and chloride

III TREATMENT —(a) *Fluids* —Saline (isotonic), glucose (isotonic and hypertonic), plasma and sodium bicarbonate solution, (b) *Cardiovascular stimulants* —(Coramine, adrenaline, strychnine, suprarenal cortical extract)

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* We are indebted to Professor M SALAH, Director of the Medical Unit, who has suggested and directed this work

Degree and Nature of Dehydration.

In 23 cases of cholera the following studies were made to find out :—

(1) The relation of clinical severity as gauged by the condition of the patient and the frequency and duration of diarrhoea and vomiting.
(2) The relation of the clinical severity to the clinical manifestation of dehydration.
(3) The relation of the degree of clinical dehydration to the specific gravity of blood and plasma.
(4) The relation of the clinical severity to blood concentration, as suggested by the haemotocrit readings and specific gravity of blood and plasma.
(5) The effects of treatment by fluids and the amount used in the various grades of dehydration (Table I).

Interpretation of the Findings.—The clinical severity was graded + ++ and +++. The clinical dehydration, as judged by the skin, the eyes, the tongue, the degree of thirst, the degree of collapse of veins and the amount of urine, was graded + ++ and +++.

1. *The clinical severity and the frequency and duration of diarrhoea and vomiting.*—In six cases grade + one showed excessive diarrhoea and vomiting, in the remaining five the attacks of vomiting and diarrhoea ranged from two to six per day their duration was from 6 hours to 4 days. In ten cases grade ++ three had excessive diarrhoea and vomiting, the attacks of diarrhoea numbered from 10 to 20 per day the duration was from 6 hours to 4 days. In severe cases, grade +++ four had excessive diarrhoea and vomiting, the duration was from 14 hours to 3 days. This shows that the clinical severity is proportionate to the number of evacuations those in which they were excessive fell into grade ++ or grade +++. On the other hand, the duration of diarrhoea and vomiting was similar in the three grades.

2. *Clinical severity and clinical dehydration.*—Table I shows that clinical severity goes hand in hand with clinical dehydration in the majority of cases suggesting that the degree of dehydration as gauged clinically is the important factor in the clinical state of the patient. In the few clinically severe cases with only ++ dehydration, the disturbances in the circulatory dynamics with marked peripheral failure can account for this difference. The amount of fluid needed to counteract the patient' clinical condition, ranged from 3 to 8 litres of isotoni saline solution. The amount of saline necessary for improvement to start is more related to the degree of clinical dehydration, and partly related to the urine and blood chlorides. This shows that the clinical severity is related to both water and electrolyte loss and not to either alone.

3. *Clinical dehydration and specific gravity of blood and plasma.*—The normal specific gravity of blood in Europeans is 1,058 and in Eastern natives 1,056 the reading is much lower in anaemic persons. The normal specific gravity of plasma ranges between 1.025 and 1,028.

The specific gravity of blood when the patient had returned to normal condition was considered as his normal figure as his red cell volume before the disease is not known. Accordingly, 13 cases showed marked increase in the specific gravity of blood, all of them showed clinical dehydration of moderate or severe degree ++ and +++. The highest rises occurred in the specific gravity of blood of cases with severe dehydration +++. On the other hand, if the figures of the specific gravity of blood on admission are studied in relation to the normal standards, we find that only six cases showed high figures. This shows that although dehydration leads to blood concentration with rise in the specific gravity the latter figures on admission do not help alone in judging the degree of dehydration this is explained by the variability of the red cell volume in these patients and the frequency of some degree of anaemia in this class of patients. Accordingly this method is more or less fallacious if taken alone as measure of blood concentration. On the other hand, if we study the specific gravity of plasma alone w find that it is above the normal standard figures in 15 cases on admission, only six of these showed higher specific gravity of blood than normal. These 15 cases with high specific gravity of plasma included all

the moderate and severe clinically dehydrated patients, as well as some with slight dehydration. Also the *specific gravity of plasma* goes, hand in hand, with the amount of plasma proteins; it is high in those cases with high blood proteins and low in cases with low plasma proteins, so constantly that the specific gravity of plasma can be taken as an indication of the amount of plasma proteins. The only two patients with low specific gravity of plasma showed low blood proteins but were severely dehydrated and died. All those with specific gravity of plasma within normal limits recovered. Five out of the 15 with high specific gravity of plasma died these were cases with severe clinical dehydration.

This shows that our clinical criteria of the degree of dehydration is parallel to the rise of the specific gravity of blood, as compared before and after treatment, and to the specific gravity of plasma on admission the latter is valuable as measure of the degree of dehydration and the amount of plasma proteins. The value of studying the plasma proteins in relation to the therapeutic use of plasma is discussed later

4 *Clinical dehydration and haematocrit*.—Only 11 cases showed higher haematocrit readings before than after treatment, *five of these were in grade + + +* while the remaining six showed only mild degrees of dehydration (+). Moreover some cases with severe dehydration showed no rise in haematocrit reading This suggests that the haematocrit reading has no value in gauging the degree of dehydration in our series of patients.

5. *Effects of treatment*.—Out of 23 patients, seven died five of these died just after arrival or at most within 12 hours and were of grade + + + clinically and in the degree of dehydration. Of the remaining two, one died of jaundice and anuria the other died on the third day in spite of intensive treatment. This shows that with intensive treatment starting early death occurred in one out of 17 cases. Even the cases showing severe clinical condition and dehydration grade + + + (four cases) were cured.

Electrolyte disturbances.—The loss of gastro-intestinal secretions by diarrhoea and vomiting results in body loss of isotonic extracellular fluid. If such a subject is deprived of all water intake, *e.g.*, by vomiting his body fluid thus tends to be hypertonic, for the insensible and sensible loss of water without salt is occurring simultaneously also, in such conditions the patient is fasting and thus the loss of cell water due to oxidation of proteins exceeds the gain due to establishment of osmotic equilibrium moreover if respiration is increased as a result of acidosis, more water vapour is lost through the lungs. As a result of this mixed depletion of water and salts, great alterations in the distribution of electrolytes and in the acid base balance take place. Osmotic *isotonicity of tissue fluids is, perhaps*, the most important of all properties in which constancy is required by the cell. Any osmotic imbalance must result in water being drawn into or forced out of the cell. In the osmotic equilibrium, between intracellular and extracellular tissue fluids, it is the electrolyte ion which plays the chief part. *If there is a greater water loss than salt loss the* extracellular fluid becomes hypertonic and as the vol me of this fluid tends to be maintained, severe dehydration occurs chiefly at the expense of the intra cellular fluid. The *cell membrane separating the* interstitial and intracellular fluids is relatively impermeable to sodium and potassium ions, the sodium being mostly outside the cell and the potassium inside Chlorine passes freely through the cell membrane but occurs mainly in the plasma and extracellular fluids. These *considerations call for* a study of the electrolyte balance in this dehydrating disease not only to detect the type of dehydration but also to guide any scientific therapeutic approach.

Results of Study of Chloride Metabolism—Estimations of blood chloride were carried out before and after treatment in 16 cases It was increased above the normal in all cases, the figures ranged from 520 to 710 mg per cent (the normal is 450 to 520) In seven cases, it showed higher figures on admission than after treatment, and in eight cases the blood chlorides were lower on admission than after treatment Of these eight cases, six were in grade + clinically and dehydration and two were in grade ++, all these eight cases improved rapidly with treatment. The seven with higher figures on admission than after treatment, fell in grade ++ dehydration and clinical, five of these showed rapid improvement, while in two the improvement was protracted but ended in recovery This shows that the higher figures of blood chlorides suggest a severer grade of dehydration and indicates more energetic treatment

Nevertheless, this estimation of the plasma chlorides is fallacious as an indicator of the total loss of chloride in mixed depletions because of the tendency to hypertonicity in the diminished extracellular fluid Thus, it is possible for the concentration of sodium ions largely in association with bicarbonate ions (and consequently the plasma osmotic pressure) to be raised when the concentration of chlorine ions is decreased In severe diarrhoea without vomiting the reverse may be true, *i e*, the concentration of the chlorine ions may be slightly decreased, or normal, or even raised, while the concentration of sodium ions is decreased

The urine chlorides were estimated in seven cases on admission The figures ranged from 1 5 to 10 5 gramme per litre In five, they were 3 5 or lower The amount of sodium chloride in the urine rose in all cases after treatment Taking into consideration the small amount of urine passed, these figures indicate marked salt depletion In comparing the urine chlorides with the blood chlorides, no correlation could be found suggesting that tissue electrolyte depletion does not reflect itself in the level of blood chlorides This also shows that blood chloride estimation is fallacious as an indicator of the total loss of these ions in mixed depletion, the urine chlorides are more useful for this purpose, and can be taken as measure of the degree of salt depletion and as a guide to salt therapy (See Table II)

Results of Study of Potassium Metabolism—The reciprocal relation of sodium to potassium in the organism is a physiological phenomenon balanced by their receptive supply and loss and by the internal control by the suprarenal glands In any condition of shock, evidence is accumulating that shifts in the electrolytes occur which increase the potassium content of the blood at the expense of tissues In a dehydrating disease such as cholera with marked disturbance of electrolytic balance, the investigation of the level of blood potassium is indicated Cellular dehydration has been found to be the one disturbance most constantly conducive to the passage of potassium out of the cells Table II shows the results of the study Also, the treatment by excessive intravenous infusions was followed in some dehydrating conditions by excessive loss of

potassium in the urine. In a recent case of diabetic coma reported by HOLLER (1946), excessive hydration was responsible for the occurrence of respiratory paralysis which was nearly fatal, were it not for the discovery of an underlying hypopotassaemia and its correction. Again there is the possibility that the suprarenal gland might be injured in such conditions. The blood potassium was estimated in 22 cases on admission. Considering that the normal blood potassium ranges from 16 to 22 mg., and contrary to CHATTERJEE and SARKAR's statement that the blood potassium increases in cholera, it was found that 18 cases showed hypopotassaemia ranging from 7 1 to 15 5 mg per cent., three cases showed figures within normal (16 to 17 1) and one case showed a higher figure than normal (22·9) this last patient died. The association of this clinically severe case, with marked peripheral failure and with a raised blood potassium, suggests the possibility of suprarenal injury as responsible for these manifestations although a postmortem could not be done to confirm this. It may also be due to severe tissue dehydration with no excretion owing to early and marked renal failure. FENN (1939) suggested that hyperpotassaemia is merely associated with compensatory transfer of cellular water to the blood stream, rather than necessarily indicative of disintegration of cells or disturbance of cell permeability In the mammalian heart, potassium acts much as it does in the frog its main effect is to promote relaxation and when present in excess it arrests the heart in diastole, and depresses A V conduction in the bundle While the mode of death does not resemble that caused by hyperpotassaemia, its depressant effect on the heart and peripheral vessels may contribute to the production of the irreversible state of shock characterised by unfavourable response to substantial infusions. This is apparently due to the fact that capillaries, especially those of the intestinal mucosa, can retain several times the normal blood volume. Thus the effective rather than total circulatory volume is reduced. In comparing the clinical severity with hypopotassaemia we found that of the 18 cases with hypopotassaemia, eight fell into grade + five into grade ++ and five into grade +++ Three of these patients died, two in grade +++ and one in grade ++ Of the 18 cases with hypopotassaemia, the blood pressure was below 100 in 11 above 100 in four and 100 in three. Again, of these 18 cases, the blood potassium rose in 11 after treatment, although still below normal. It was further diminished in five cases. No relation was found between the amount of glucose given and the changes in the blood potassium. This point will be further discussed under treatment. This shows that hypopotassaemia has no relation to the degree of the severity of the clinical condition. Of the six patients who died the blood potassium was reduced in three, normal in two, and in one, increased. It can also be seen that saline and glucose infusions were not sufficient to raise the blood potassium to normal. The following ill-effects of hypopotassaemia are available in the literature alterations in the electrocardiogram in hypopotassaemia of familial periodic paralysis were reported by STEWART (1940), KEH WEI HUANG and

TABLE II

Case number	Clinical severity	Clinical dehydration	Blood pressure	Venous pressure	Before treatment: Specific gravity blood.	Before treatment: Specific gravity plasma.	Before treatment: Urine, c.c	Before treatment: Urine-chloride g per litre NaCl	Before treatment: Blood chloride mg % NaCl	Before treatment: Blood urea mg %	Before treatment: Blood potassium, mg %	After treatment: Urine chloride, g per litre NaCl	After treatment: Blood chloride, mg % NaCl	After treatment: Blood urea, mg %	After treatment: Blood potassium mg %	Remarks.
1	++	+	0	1	1,046	1,023	Nil		710	44	16	7 1	555	16	12 1	
2	++	+++	100/60	3 5	1,071	1,036	100	3 5	520	68	15 1	6 2	780	27	14 1	
3	++	+	60/40	3 5	1,055	1,025	100	2 1	610	58	12 3	12 5	570	19	15 2	
4	+	+	100/70	7	1,055	1,010	100		600	41	10	1 2	615	18	15 1	
5	++	++	100/60	4	1,056	1,035	100	1 5	520	179	9 5	4 5	580	16	11	
6	+++	+++	0	1	1,051	1,024	100		590	32	15 6	1 9	585	140	11 7	Azotaemia
7	+++	+++	0	1 5	1,041	1,021	100	5 5	605	20	17 1		770	191	11 3	Died
8	+	+	120/80	5	1,053	1,033	500	3 5	620	160	14 2	4 5	740	21	9 9	
9	+	+	110/80	7	1,059	1,037	100		610	29	12 1	7	800	24	15 1	
10	++	+	60/30	3 5	1,063	1,033	100		610	20	13 8	5 5	535	20	12 1	
11	+++	+++	0	0	1,069	1,035	Nil				22 9					Died
12	++	+++	0	0	1,057	1,033	Nil				10					„
13	+++	+++	0	0	>1,074	1,017	Nil				7 1					„
14	+++	+++	0	0	1,074	1,059	Nil									„
15	+	+	110/70		1 058	1,027	100	10 5	505	31	12 1	11 5	575	18	12 4	
16	++	++	0	3	1 058	1,033	Nil				12 8				16 8	Died
17	+	+	130/80		1,064	1,036	65		570	58	12 4	7 5	610	28	13 5	
18	++	++	0		1,050	1,029	100		615	22	13 0	12 8	570	18	14	
19	+	+	80/70		1,053	1,026	500	2 2	545	105	13 5	5 1	570	30	14 4	
20	++	++	70/40		1,063	1,040	Nil		630	118	14 0	5 7	590	19	12 5	
21	+++	+++	0		1,062	1,017	Nil				17 1					Died
22	++	+++	30/0	1 5	1,065	1,037	Nil		680	72	12 8	3	400	147	15 2	Azotaemia
23	+++	++	0		1,057	1,032	100			52	12 1	12 5	580	17	14 6	

YING-CHI MOA (1947) reported a paralytic hypotonic syndrome in cases of cholera and suggested owing to its similarity to familial periodic paralysis that it is related to hypopotassaemia, without confirming this suggestion by blood potassium estimations. In spite of the frequency of hypopotassaemia in our cases and the markedly low figures of blood potassium met with in some of them, no such paralytic manifestations were encountered although particularly looked for The explanation of hypopotassaemia in these cholera cases needs further investigation It may possibly be due to loss of potassium by the excreta in the absence of any intake of potassium estimations of potassium contents of excreta of cholera cases are indicated to substantiate this suggestion.

Plasma Proteins and Dehydration.—The severe loss of fluid leads to a rise in some of the constituents of the blood, especially proteins. If the inflammation of the gastro-intestinal mucosa is sufficiently severe capillary damage results in protein loss. This counteracts the rise in plasma proteins produced by blood concentration. The sum of the two factors should indicate which of them is more prominent In the cases where this second factor is more prominent, a rise in plasma proteins due to blood concentration, will be prevented

The plasma proteins were estimated in 23 cases 16 showed hyperproteinaemia ranging from 7 53 to 17·4 gramme per 100 c.c. of blood, while seven cases showed hypoproteinaemia ranging from 3·43 to 5·65 gramme. In these seven cases hypoproteinaemia occurred in spite of severe dehydration +++ in three and of moderate dehydration ++ in two, suggesting a definite reduction in the plasma proteins. Is the plasma protein deficit in these cases (one third of the cases) due to excessive protein loss, interference with plasma protein formation by the liver or nutritional ? The hepatic factor is certainly improbable or only minor as such low figures of hypoproteinaemia could only result from very severe hepatic injury together with liver failure. Whatever the explanation of the reduction might be, the fact remains that these patients need restoration of their plasma proteins to normal by plasma infusions. The definite correlation of the figures of the specific gravity of plasma with those of plasma proteins give the former its value as an indication for plasma infusions not only when low but also when normal as this normality is apparently due to blood concentration. (Table I.)

Azotaemia and Dehydration.—In the presence of a normal kidney dehydration is known to lead to prerenal accumulation of nitrogenous products, failure of their excretion is brought about by a disturbance in the availability and distribution of water bases, chlorine and bicarbonates needed by the kidneys for proper functioning. As a result of dehydration, the effective circulating blood diminishes, leading to diminished venous return to the heart and fall in the cardiac output, thus reducing blood flow to the organs, including the kidney Also as a result of hypochloraemia, if present, renal elimination of toxic waste products becomes increasingly difficult.

BANERJEE and DUTTA (1945) have shown in fatal cases of cholera, foci

of necrosis in the glomeruli and marked changes in the convoluted tubules in which casts were found CHATTERJEE (1945) showed that acute inflammatory changes were absent as a rule, although acute congestion in the medulla and glomerular capillaries might be seen The changes are more marked in uraemic kidneys than in the non-uraemic ROGERS (1939) states that it usually takes 80 to 100 mm or more of mercury pressure to force saline fluid through the renal blood vessels after death from cholera, whereas 20 to 30 mm are enough in persons dying from most other diseases This shows that during the collapse stage the great congestion of the kidney inpedes blood circulation through it, and thus may account for the suppression of urine during this stage MOON (1947) concludes that in shock the azotaemic manifestations are due to tubular dysfunction

In 17 cases, the blood urea was estimated before and after treatment , it was high in 11, the figures ranged from 41 to 179 mg per cent , taking into consideration the fasting state of the patient In all cases the blood urea returned to normal after treatment except in three, where it rose, in only one of these was anuria present The blood urea figures do not bear any special relation to the degree of clinical severity The azotaemia is also not quantitatively related to the degree of blood concentration Although BANERJEE suggests that hypochloraemia is a factor in the production of azotaemia, our findings do not support this view as no case with definite hypochloraemia was found, although salt depletion of tissues as shown by urine chlorides, is demonstrated

Although dehydration per se leads to prerenal azotaemia through tissue disintergration and circulatory failure with oliguria, a renal factor is suggested to operate in addition in these cases owing to the insignificant role played by the above factors in some of the patients with high figures , also the renal factor is supported by the low specific gravity of urine found in some of these cases, in spite of the scanty volume passed This renal damage of rapid development suggests that toxaemia may play a part in its production

It is convenient here to discuss the mechanism, effects and prognostic significance of anuria and the importance of its rapid correction The amount of urine was diminished markedly in all cases In only two, the amount of urine was about 500 c c In 12 cases it was about 100 c c , and in nine cases anuria was present The eight anuric cases fell into grade ++ and +++ (See Table II) This shows that the anuria is partly related to the degree of clinical dehydration, but circulatory factors, especially the venous pressure, also play an important role Analysis of data also suggests a renal element in the production of anuria as well as azotaemia , we have previously pointed out that the latter is not mainly the result of blood concentration or anuria The prognostic significance of anuria can be seen from studying the cases of the seven patients who died Of these, six were anuric on admission, and the seventh developed anuria later on One developed anuria and uraemia with diminution of urine

chlorides during treatment but was saved. This shows that anuria is a serious prognostic sign, indicating energetic correction of the factors discussed as responsible for its production.

Blood Sugar and Dehydration.—The starving condition of cholera patients during the active period suggests studying their blood sugar to see whether their treatment should include glucose administration or not. The factor of blood concentration is taken into consideration, and for this reason the blood sugar estimation was repeated after saline transfusion. (Table I.)

The blood sugar was estimated in 21 cases on admission it was lowered in two within normal in four cases, and definitely increased in 14 cases, ranging from 143 to 280 mg per cent., and in one case the figure was 565 This patient had the highest degree of blood concentration and died within 24 hours. The degree of hyperglycaemia corresponds more or less to the degree of haemoconcentration as measured clinically and by the specific gravity of plasma also it is known that hyperglycaemia occurs in cases of circulatory failure 12 of these cases with hyperglycaemia showed marked lowering of the blood pressure, and the blood sugar was shown to rise with the deterioration of the clinical condition, including the circulation in three cases under treatment. CHATTERJEE and SARKAR (1941) have pointed out that lowering of the blood sugar may occur in cholera. This was met with in only two cases. After correcting the dehydration and recovery of the clinical condition, all blood sugars returned to normal or low normal (80 to 134) in one case it was 65 mg per cent. As to the reason why the blood sugar did not rise in spite of blood concentration in six cases, one may speculate that these patients store of glycogen in the liver and muscle is so depleted that no rise in blood sugar occurs they all rose to normal figures after treatment with saline and glucose. The amount of the latter was not particularly greater than had been given to other cases with hyperglycaemia. Anyhow a normal or low blood sugar calls for earlier glucose transfusion to correct this deficiency

SUMMARY AND CONCLUSIONS OF THE FOREGOING STUDIES.

I Dehydration is the main process governing the degree of clinical severity of cholera cases. The grading of dehydration, as measured by the clinical criteria mentioned, is as efficient in this respect as delicate laboratory procedures demonstrating blood concentration, the best of these is the specific gravity of plasma.

II. Although the degree of dehydration depends mainly on the number of bowel evacuations rather than their duration yet it appears that some patients are more easily dehydrated than others. This gives the clinical measure of dehydration, as outlined here, more significance in calculating the amount of fluid needed for its correction. Clinical dehydration is the result of both blood concentration and tissue dehydration.

III. The clinical results of dehydration are due to loss of both water and

salts, the former is more important in this respect as no special clinical manifestations could be ascribed to salt depletion alone This statement indicates the use of isotonic saline with or without glucose in the treatment of these cases and gives no support to the use of hypertonic saline

IV Toxins seem to take part in the clinical manifestations of this disease mostly in relation to circulatory failure and anuria In addition, as a result of this work, toxaemia has also to be blamed as partly responsible for some of the biochemical changes found, *e g*, azotaemia

V The following physico-chemical changes were found in the cases of cholera studied and their mechanisms discussed

1 *Specific gravity of blood*—Most of the cases with moderate or marked dehydration showed definite rise in the specific gravity of blood (13 out of 23) Because the figures on admission were higher than normal in only six of these cases due to associated anaemia, a single determination before treatment is considered unreliable as a sign or measure of the degree of blood concentration

2 *Haematocrit value*—The same fallacy applies here Only three showed higher figures than normal on admission owing to variability of the red cell volume and the frequent occurrence of some degree of anaemia in this undernourished class of patients

3 *Specific gravity of plasma*—All the cases with clinical dehydration showed higher specific gravity of plasma on admission than normal (15 out of 23), only six of these showed high specific gravity of the blood This illustrates the value of this determination as an index and measure of the degree of blood concentration

4 *Plasma proteins*—Hyperproteinaemia is evident in two-thirds of cases, obviously the result of haemocentration In the remaining one-third, hypoproteinaemia, probably the result of protein loss, was demonstrated Plasma protein determination is of value for indicating plasma transfusion, the parallelism of the plasma specific gravity with the amount of plasma proteins shows that the former is of value, being simpler, for this determination

5 *Blood urea*—Azotaemia was demonstrated in two-thirds of the cases studied, it has no relation to the clinical severity, it disappeared in all but three cases under treatment Although it is mainly related to dehydration and circulatory failure, it has no relation to the degree of blood concentration, or hypochloraemia A renal factor of toxic nature in the production of azotaemia is suggested by the present work

6 *Blood sugar*—Hyperglycaemia was evident in more than two-thirds of the cases, its degree corresponds with the degree of blood concentration which is the main factor in its production Hypoglycaemia was present in only two cases

7 *Blood potassium*—Contrary to previous statements diminution of blood potassium was demonstrated in the majority of cases (18 out of 22) It bears no relation to the clinical severity, and it was not responsible for any special clinical manifestation, no hypotonic phenomenon was found It is probably due to excessive potassium loss in the absence of intake The saline-glucose treatment was not sufficient to raise the blood potassium in these cases to normal

8 *Blood and urine chlorides*—The blood chlorides were markedly increased on admission in all cases examined (16 cases) The higher the figures of blood chlorides the more the degree of dehydration This hyperchloraemia occurred in spite of salt depletion and thus is unreliable as an evidence of chloride disturbance Contrary to previous statements no hypochloraemia was found On the other hand, the urine chloride estimations show their value as a definite indicator of the presence and degree of tissue salt depletion so long as the kidney function is not appreciably impaired The urine chlorides were markedly diminished in all cases examined, taking into consideration the amount of urine passed, it was 3 5 gramme per litre or less in the majority of cases We feel that urine

chloride estimation is not only of value as measure of the amount and urgency of saline infusions but also guides the progress of saline treatment.

Prognosis in Cholera.

(1) The degree of dehydration and its *duration, and the availability of* proper energetic treatment. If severe and prolonged, dehydration may lead to irreversible cellular damage in addition to the effects of toxaemia and anoxaemia.

(2) Anuria is bad prognostic sign needing energetic treatment, even then six out *of nine cases* of anuria did not respond to the above treatment and died.

(3) The degree of disturbed circulatory dynamics as measured *particularly* by the venous pressure patients with very low or unmeasurable venous pressure are bad risks.

TREATMENT RECOMMENDED.

Treatment should start immediately the patient *is seen even at home.*

1 Avoid any effort during the transfer of the patient, immobility in bed should be *insisted* upon, even *during* defaecation and vomiting.

2. Warm the patient with blankets or hot water bottles for short time. Energetic heating by electric baths, etc., is condemned.

3. Bandage the limbs as first-aid measure

4. Nothing by mouth until the gastro-intestinal irritation is alleviated.

5 Stimulants.—Coramine is given as soon as the patient is seen. The use of the other stimulants is left until the clinical examination decides their indications.

6. Careful nursing is of paramount importance contiuous and thorough observation of the patient condition is essential.

7 Fluid therapy —This should be started immediately the patient is seen together *with the preliminary supporting treatments. Fluid administration if graded precisely* (in quantity and quality) in the light of combined sound clinical judgment and adequate *repeated laboratory data, gives marvellous results,* while insufficient or improper use of fluids leads to grave consequences. The guides to proper fluid administration as regards *quantity and quality are* :—

( ) The degree of dehydration, clinical grading mentioned in this work is sufficiently accurate.

(*b*) Blood pressure estimation gives us a preliminary idea of the degree of disturbances in the circulatory dynamics, and whether compensatory vasoconstriction is present or not. But as shown in another paper estimation of the enous pressure is the most accurate criterion of the affection of the circulatory system and thus the lower the venous pressure the more energetic the treatment should be.

( ) The degree of blood concentration estimation of the specific gravity of plasma is the most accurate, simple and rapid method for this purpose The more the blood concentration the more rapid infusion is indicated to correct blood volume, also the specific gravity of plasma indicates whether plasma is specifically needed or not if low or normal, plasma transfusions are indicated.

(*d*) *The amount, reaction and chloride content* of the urine collected 8-hourly This procedure gives information on the degree of dehydration, the presence or *absence of acidosis,* and the *degree of* salt depletion acidosis indicates alkali administration, while the amount of chlorides in repeated samples governs the nature (isotonic *or hypotonic*) and the amount of saline solution to be continued.

( ) Blood urea estimation.—Azotaemia indicates restriction of proteins and energetic correction of the blood volume and the disturbed circulatory dynamics repeated estimations are needed to guide the continuation of these measures.

The Amount of Fluid Needed —This depends mainly on the degree of dehydration as *measured* clinically and by the specific gravity of plasma denoting

the degree of blood concentration, these usually go hand in hand MADDOCK and COLLER believe that the presence of clinical signs of dehydration indicates a loss of 6 per cent of body weight, thus a man weighing 70 kg may be assumed to have a negative balance of 60 c c per kg body weight, or a total of about 4,200 c c, thus 60 c c of fluid are to be given per kg body weight per day to this patient In the cases studied in this work we found the amount of fluid needed according to the criteria mentioned is as follows

Dehydration grade	Sp Gr of plasma	Fluid needed per 24 hours
+	1025–1030	6 to 8 litres
++	1031–1040	8 ,, 10 ,,
+++	1041–1050	12 ,, 14 ,,

These amounts are higher than those of MADDOCK and COLLER (1945), because we aim at supplying the daily requisite plus the amount of fluids lost by the persistent evacuations, the larger amounts are given to those with more frequent vomiting and diarrhoea

The Route of Administration—The intravenous route is the ideal way of administering fluids, oral and rectal routes are obviously useless, moreover, if fluid could be administered by these routes stimulation of the irritated gastro-intestinal tract follows Fluids administered subcutaneously are not absorbed in the presence of circulatory failure, moreover, the amounts given are too high for subcutaneous introduction it can be resorted to later, after water and salt balance is corrected for maintenance Intraperitoneal administration of fluids, as tried in some cases, was found to be rather difficult in the presence of the sunken abdomen for fear of injuring viscera

The intravenous route was possible in almost all cases, no incisions to expose veins were resorted to except occasionally The arm, leg and jugular veins were usually available for repeated infusions

In the few cases in which repeated intravenous administration was not possible, as well as in some others for comparison (10 cases), we gave the infusions intramedullary through a sternal puncture needle, which method, in addition to its simplicity, if carried out by an experienced person, proved of great value as a rapid and efficient way of fluid administration We recommend it to save time if veins are difficult to find The fluid administered should be at body temperature

The Frequency of Fluid Administration—We recommend the following scheme

(*a*) The first 2 litres are administered quickly, within a period of half an hour This is meant to correct the diminished blood volume as quickly as possible

(*b*) This is followed by a litre of fluid every 2 to 4 hours (administered by moderately quick drip) according to the criteria previously mentioned and the calculated total fluid needed within 24 hours

(c) Fluid administered should be continued so long as —

(i) The venous pressure is low or veins still *collapsed*.
(ii) The patient is still thirsty and the tongue is still dry with persistence of other signs of dehydration.
(iii) The 8-hourly urine is still below 400 c.c. in quantity
(iv) *Azotaemia is still present.*

The amount and frequency of administration should vary according to the degree of variation of values given above so as to avoid over hydration and pulmonary oedema the latter should be looked for repeatedly

The Nature of Fluids Administered.—Fluids administered should contain the ingredients we found missing in these cholera patients. We have shown that dehydration of cholera patients is a mixed one of salt and water depletion the latter is, more than the former and accordingly hypertonic saline treatment suggested by ROGERS, *is contra-indicated not only because of the less need* for salt than water but also because of the hypertonicity of tissue fluid the increase of which increases cellular dehydration. Scientifically hypotonic *saline solution is indicated in this type of dehydration*, but to replace salt depletion as quickly as possible to correct electrolyte imbalance, we recommend starting the treatment with isotonic saline solution. As a result of our findings of the frequency of hypopotassaemia, Ringer's solution seems to be more scientifically indicated to correct the combined electrolyte deficiencies. This solution should be continued according to the lines given above as regards amount and frequency until the urine chlorides reach 5 gramme per litre or more this indicates the replacement of the essential basal amount of sodium chloride needed for maintaining a more or less normal electrolyte balance. When this stage is reached the solution is made hypotonic by the addition of an equal amount of normal glucose solution (5 per cent.). This latter also supplies nutrient calories, sparing protein destruction, diminishes the tendency to starvation acidosis and helps diuresis in this stage to rid the blood of azotaemic products. This combination (Ringer + 5 per cent. glucose in equal parts) should continue so long as parenteral fluid administration is needed and until hydration is approached, continuation of such hypotonic (saline) solution after this stage may lead to pulmonary oedema thus if fluid administration is still further needed *for correcting any persisting* disturbed circulatory dynamics, azotaemia or oliguria, hypertonic glucose 25 per cent. should replace the normal glucose in a proportion of 1 3 to 2 3 Ringer's solution until these disturbances are corrected.

Sodium lactate is the best alkalinizer to administer to correct acidosis its use is not associated with the tendency to change the reaction to marked alkalosis, as with sodium bicarbonate, and there is no need to follow the changes in the alkali reserve to avoid this shift. Alkalosis is deleterious to the patient especially to his renal functions, which we aim to correct. Accordingly if signs of acidosis are evident, the reaction of the urine and the presence of air hunger type of respiration, were sufficient criteria for its detection, the sodium lactate

can be added to this Ringer's solution or combination at any stage, in the proportion of 25 ml of con (molar) sodium lactate solution to each litre This can be continued until the reaction of the urine becomes neutral or slightly alkaline and then stopped

Plasma transfusion—Only a solution containing colloids can reconstitute the volume of the circulating blood In this respect, blood plasma is the best as at the same time it corrects hypoproteinaemia if present, and supplies specific and non-specific antibodies Accordingly, plasma transfusion, if available, is ideal and should be administered as early as possible at the beginning of treatment with the Ringer solution in doses of 500 c c, which can be repeated two to three times, as indicated On the other hand, plasma transfusion is not usually available in sufficient amounts for every case In such circumstances, we feel that plasma is particularly indicated with the fluid infusions and should be resorted to in the two following conditions Very low arterize and venous pressure, normal or low specific gravity of blood plasma.

Both human plasma and calf's plasma prepared by BARSOUM (1948) were used, the latter was followed by some reactions (fever and urticarial rash)

8 *Specific Treatment*—Although some agents affected cholera vibrios *in vitro*, their therapeutic value in cholera cases is not agreed upon

(*a*) Sulpha drugs Studies by GRIFFITTS (1942), *in vitro* and in mice revealed that sulphathiazol, sulphadiazine and sulphaguanidine are active in inhibiting the growth of cholera vibrios The epidemiology unit No 50 studying the treatment of cholera (in Calcutta) came to the conclusion that the most striking effects appeared to be due not to chemotherapy but to plasma transfusion, nevertheless they believe that chemotherapy in addition to hydration treatment lowers the death rate for cholera This and other reports show that chemotherapy by sulpha drugs is justified as a clinical experiment It appears to us that sulphaguanidine is the most suitable for this purpose because of its marked local effect and little absorption, soluble absorbable sulpha compound may, in these oliguric patients be harmful and enhance or aggravate the renal dysfunction This compound was used in our cases in addition to the above treatment, it was noticed that if the drug stopped early (after 3 days only) some diarrhoea may reappear, to disappear again on its readministration, therefore it is advisable to give it for at least 1 week (better 10 days)

(*b*) *Streptomycin*—Although certain strains of the cholera vibro are highly sensitive to streptomycin in the test tube, REIMANN (1946) believes that it has no specific effect in the treatment of cholera As the oral route may be of some effect, we tried streptomycin by injection and orally in some of our cases, the results will be discussed in another paper

(*c*) *Antitoxic sera*—As a supplement to sulphaguanidine, it proved to be effective in severely toxic cases No cases of uraemia appeared in the series in which GHOSH (1936) tried anti-cholera serum It was not available for the present study although its clinical trial is suggested

9 *Treatment of complications of cholera*—The following complications occurred in the present study (42 cases)

(*a*) Otitis media in two cases recovered under penicillin and local treatment

(*b*) Pneumonia in three cases (one case followed pulmonary oedema) They were cured by penicillin

(*c*) Jaundice in one fatal case

(*d*) Severe gastro-intestinal haemorrhage in one patient with cirrhosis of liver, saved by repeated blood transfusions

(*e*) Abortion in five, these showed marked improvement later

(*f*) Morbilliform rash appeared in three cases, these did not receive plasma.
(*g*) Tetany in female patient appeared three days after stopping the infusions, which contained no alkali, it recurred for 3 days and each time it disappeared after calcium injection intravenously

No sequelae were met with in this series observed for a period of 3 to 4 weeks.

REFERENCES.

BARBOUM, H. (1948). *Lancet* 1 348.
BANERJEE, D N & DUTTA, S. K. (1945). *Manson Tropical Diseases.* 12th Ed. 453 London Cassel & Co., Ltd.
CHATTERJEE, H. M. (1945). *Stitt' Tropical Diseases.* 7th Ed. 1 613–619 London H. K. Lewis & Co.
—— & SARKAR, J (1941). *Trans. R. Soc. trop. Med. Hyg* 34 379
FENN W D *et al.* (1939). *Amer J Physiol.* 128 139
GHOSH, H (1936). *Brit. Med. J* 1 936.
GRIFFITH, J J (1942) *Pub. Hlth. Rep* 57 814
HALLER, J W (1946). *J Amer med. Ass.*, 131 1186.
KEH WEI HUANG, *et al.* (1947). *Amer J med. Sci.*, 2 214
MADDOCK, W G & COLLER, S. A. (1945). *Dietotherapy by Wohl.* 1st Ed. 908 London Saunder Company
MOORE, V H. (1947). *J Amer med. Ass.*, 134 425.
RIEDEMANN, H A. (1946) *Ibid.*, 132 183
ROGERS, L., & MEGAW J W D (1939). *Tropical Medicine* 5th Ed. 273 London: Churchill, Ltd.
STEWART H. J (1940). *Amer J med. Sci.*, 199 789

TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE
Vol 43 No 1 July, 1949

CORRESPONDENCE.

ABNORMAL FORMS OF *PLASMODIUM VIVAX*

To the Editor, TRANSACTIONS *of the Royal Society of Tropical Medicine and Hygiene*

SIR,

We have only recently had an opportunity of reading Dr J W FIELD's paper on Morphological Variation in *Plasmodium vivax* which appeared in *Parasitology* **34,** 82, and was reviewed in *The Tropical Diseases Bulletin*, **40,** 7 This account of abnormal forms of *P vivax* interested us very much, as the parasite descriptions resemble in some points parasite infestations that we observed in three cases infected in the Moshi district in 1937 We, unfortunately, had no clinical notes on the cases as they had already commenced treatment at the time of examination of the blood films, but, morphologically, they resembled those described by ROBERTS (1941), and named *P wilsoni* *East African Med Journal*, **17,** 215

The points of resemblance between them, made at the time from notes and camera lucida drawings, and those described by Dr FIELD are

(1) Multiple invasion of single red cells with up to four parasites

(2) Distortion of the host cell (many were quite astonishingly distorted)

(3) Compactness of the smaller parasites

(4) Closely packed schizonts

(5) Resemblance of parasites to *P malariae* except for their presence in enlarged cells

The points of difference are as follows

(1) The corpuscle was always enlarged, sometimes very greatly so, and paler than normal

(2) Stippling of the cell either absent, or if present, of a faint *P malariae* type, or trabeculation of the cell

(3) Parasites occasionally seen which were separated from the corpuscles in which they had grown

(4) The multiple parasites in single cells were practically never vacuolated rings, but were of varied form and size. The chromatin often appeared separate from the cytoplasm, and in some cases the impression received by the eye was that of various little pieces of chromatin and cytoplasm dotted about the greatly enlarged cell.

It is probable that the parasites described by ROBERTS (1941) were abnormal forms of *P. vivax* but whether the original parasites we observed in 1937 and demonstrated at a meeting of this Society in 1938, were also abnormal forms of *P. vivax* is more doubtful.

We continue to hope to encounter them again and have the opportunity of making repeated examinations and parasite drawings, together with a complete clinical history.

I am, etc.

MARGARET WILSON, M.B., D.T.M.

Malaria Laboratory,
Muheza,
Tanganyika Territory,
East Africa.

MALIGNANT MALNUTRITION

SIR,

I was very interested in the paper by Dr H C TROWELL on "Malignant Malnutrition"

A little over 30 years ago I encountered malnutrition in the prisoner-of-war camps in Germany, especially in Lamsdorf There was no tropical disease, but depigmentation of hair was to be seen, and the summer of 1919 produced the dermatitis

Later, in the Bukoba District of Tanganyika Territory, I saw and attempted to record a food deficiency disease, and thought it was pellaginous, associated with protein lack A few years ago, in India, one had only to walk amongst the population to see much evidence of malnutrition It is to be seen in this country in the milder form

It seems to be accepted that classical pellagra "is rare in the tropical regions of the world" That may be so, but I saw several beautiful "necklaces of Casal" in Iringa, Tanganyika Territory

It is all very interesting, and it seems strange that we are about to approach these poor areas for some of our foods Perhaps a few crumbs will fall from the table to help my former friends of the Tanganyika Territory

I am most grateful to Dr TROWELL, and wish I could have been present at the discussion

I am, etc ,

JOHN HARKNESS

24, Hermitage Gardens,
Edinburgh
23rd April, 1949

PROCEEDINGS OF THE FOURTH INTERNATIONAL CONGRESSES OF TROPICAL MEDICINE AND MALARIA

WASHINGTON, 1948

Corrigendum

SIR,

May I request the hospitality of your pages to correct a grave typographical error in my communication on "The Relationship of the Haemoflagellates," which appears in Vol. 2, page 1110, of the *Proceedings of the Fourth International Congresses of Tropical Medicine and Malaria*, Washington, 1948. (Published in 1949.)

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Corrigendum. On page 1113, line 22 should be transferred to line 39 and *vice versa*.

I am, etc.,
C. A. HOARE.

Wellcome Laboratories of Tropical Medicine,
London.

May 25th, 1949.

CORRIGENDUM

Dietary deficiencies in children in the island of Viti Levu, Fiji by F. A. Thomson. Vol. 42, p. 487.

Photograph 7 represents the upper jaw and should be reversed.

ANNOUNCEMENTS.

NEXT MEETING OF THE SOCIETY

The next meeting, the Opening Meeting of the 43rd Session, will be held at Manson House on Thursday, 20th October, 1949, at 7 30 p m Professor H E SHORTT, C I E, M D, will deliver his Presidential Address, entitled "Tropical Medicine as a Career"

MOVEMENTS OF FELLOWS

The following Fellows from abroad have notified the Secretaries that they are *temporarily* in the British Isles Letters addressed to any of these care of the Royal Society of Tropical Medicine and Hygiene, Manson House, 26, Portland Place, London, W 1, can usually be forwarded to the home address

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WRIGHT, E J, Sierra Leone
YEO, K C, Hongkong

NEW HONORARY FELLOWS

Elected at Council 16th June 1949

M j -Gen. A. J Orenstein, South Africa.
Dr Paul F Russell, U.S.A.
D Wilbur Sawyer, U.S.A
D N H Swellengrebel, Holland
D W H. Taliaferro U.S.A.

NEW FELLOWS

At the meeting of the Society held at Manson House on 16th June 1949 the following 44 candidates were elected Fellows of the Society —

Abdultam Aboulwahab Z., M.B., CH B. (CAIRO), D.T M & H (ENG.), Sudan.
Ali Mohamed Ahmed, M.B.E., Diploma, Kitchener School of Medicine Sudan.
Anis, Ibrahim, Diploma, Kitchener School of Medicine D /Asst. Director Sudan Medical Service
Baidya, Dinobhanandа, M.B. (CAL.) India.
Banerjee, Durgadas, M.B. (CAL.), India.
Bhattacharji L. M D., D.T.M., D P H. (CALCUTT), India.
Brotien, Otto C., M.D (TUFTS MED. COL.), U.S.A.
Brown Alexander, M.B., CH B. (EDIN.), F.R C.P.E., Nigeria.
Calderaquin, Prudencia B. M.D. (MANILA), Philippines.
C mpbell, A. E., M.D. (BELFAST), D.P.H (DUBLIN), Colonel, late R.A.M.C.
Chen Tsu Tu M.D. (YALE MED. COL., CHINA) D.T.M. (CALCUTTA), China.
Chuchin, Li, M.D. (NANKING, MILITARY MED. SCHOOL), China.
Das, Gour Mohan, M.B D.T.M., D.P.H (CAL.), Asst. Malariologist, West Bengal.
Francisco F S., M.D. (PHILIPPINES) Philippines.
Gibson Robert Smith, M.B., CH B. (GLAS.), Southern Rhodesia.
Hakim, Hamamel, D.K.S.M., Sudan Medical Service
Hayman, Andrew P M.D., Col Med. E angeles, (Calif.) U.S A.
Hirsch, Hans, M.B., B.CH. (WITW TERSRAND), South Africa.
Hsu, Yu Mien, M.D B.SC. (SHANGHAI), Hongkong
Jachowski, Leo A., B.S., M.S. (MICHIGAN) Lieut. M.S.C., U S. Navy
Jaikaran Lionel B M.R.C.S. (ENG.) L.R.C.P (LOND.), British Guiana
Khorram, M. A., M.D. (BERLIN) Iran.
Koo Pei Kai, M.D. (NANKING), China.
Lawrence, David, M.B. B.S. (LOND.), England
McQuade, Henry Grey M.A. M.B., B CHIR. (ANTAB.), M.R.C.S. (ENG.) L.R C.P (LOND.) Nigeria.
McQuay, R. Michael, M.S., PARASIT (TUL NE), U.S.A.
Marsh, A. Howard M.B., CH B. (N.Z), D.M.R., Royal N.Z. Air Force, New Zealand.
Mirghani, M. A., DIP SC (KHARTOUM), Vet. Research Officer Sudan.
Montgomery J H., M.B., CH.B. (EDIN), Hongkong
Moore, Diedrik, M.B., CH.B. (CAPETOWN) South Africa.
Owen Gwynfryn A., M.R.C.S. (ENG.), L.R.C.P (LOND.) Gold Coast.
Park, Robert M.R.C.S. (ENG), L.R.C P (LOND.), Nyasaland
Rendtorff Robert C., D.SC. (JOHNS HOPKINS) U.S.A
Rizzolo, John M.D., B.S. (Maryland) U.S.A.
Ross, Serge Gregory M.D. (KHARKOV), Fiji Islands.
Sompranes, M G M D. (MEXICO) Degree Epidemiologist, U.S.A.
Spitz, A. J Wilhelm, M D (VIENNA) D.T.M. & H (ENG.), England
Stewart Ian M. S., M B CH.B. (ABERDEEN), South Iran.
Subramanian R M.B B.S (MADRAS), D P.H (CAL.), India.
Tambimuttu J R C L.M.S. (CEYLON), Ceylon.
Thomson Ian Gordon M.B CH.B. (ABERDEEN), Nigeria
Toskas, Vasilios D M (ATHENS) U.S.A.
Wilson, W C., M.B B.S. (LOND), Southern Rhodesia.
Woolfe, Gerald, M SC PH D. (M NCHESTER) *England (Boots Pure Drug Co)*

ADMISSION TO FELLOWSHIP OF THE SOCIETY

All registered medical and veterinary practitioners and others interested in scientific pursuits relating to tropical medicine, whose qualifications are deemed satisfactory by the Council, are eligible for election as Fellows of the Society

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Further information may be obtained from the Hon Secretaries, Manson House, 26, Portland Place, London, W 1 , or from the Local Secretary of the district

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When copies of the TRANSACTIONS are returned by the Post Office marked "Gone Away," "No Service," or "Insufficient Address," no more copies will be posted to that address but they will be retained at Manson House until further instructions are received

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NEW BOOKS RECEIVED

Advances in Internal Medicine Vol III By WILLIAM DOCK and I SNAPPER New York and London Interscience Publishers Price 51s

Proceedings Fourth International Congresses on Tropical Medicine and Malaria Washington 1948 Vols I and II Department of State, Washington, D C

From the Society's file of the *Annales de la Société Belge de Médecine tropicale*, No 1 of Volume 19 (1939) is missing, and is not obtainable from the publishers If any Fellow has a spare copy, it would be gratefully received at Manson House

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FOURTEENTH INTERNATIONAL VETERINARY CONGRESS

At Central Hall, Westminster, London, S W 1, and in Church House, Westminster London S W 1, on the 8th to 13th August, 1949

Further particulars from the Organizing Secretary, Fourteenth International Veterinary Congress, 10, Red Lion Square, London, W C 1

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Papers should, if possible, be typewritten; they should be concisely written with subject matter logically arranged and sub-divided; with references and abbreviations in the form described below; and with indications of the position, in the text, of illustrations, tables, maps, etc.

Titles should be as brief as consistent with clarity; and in many cases the value of a paper is enhanced by a short **summary** at the end.

Temperature charts, graphs and drawings should be, if possible, in Indian ink on Bristol board, with detail and essential lettering large enough to be clearly legible after reduction if necessary. (Write in pencil if lettering on a drawing is to be set up and printed.)

Illustrations—if the number sent in is considered excessive, the author may be informed and given the opportunity of contributing to the cost.

Coloured plates are made only at the authors' expense.

REFERENCES.

In the text, the date of publication, in brackets, should follow the name of the author quoted, thus:—

T. MANSON (1879) made this epoch-making discovery.

At the end of the paper, a list of **References** should be arranged in alphabetical order of authors' surnames, and details given in the following order:—(1) Surname of author; (2) Initials of author; (3) Year of publication in brackets; (4) Title of article avoiding arbitrary capitals. (The title of the article is sometimes omitted; but each list of references should in this respect be consistent throughout—giving all titles, or omitting all.) (5) Title of journal; (6) Volume number; (7) Page number, e.g.:—MANSON, P. (1879). On the development of *Filaria sanguinis hominis* and on the mosquito considered as a nurse. *J. Linn. Soc. (Zool.)*, 14, 304.

In the case of a reference to a **book**: (1), (2) and (3) as above; (4) Title of book; (5) Edition and/or volume, if more than one; (6) Page number; (7) Town of publication; (8) Publishers' name, e.g.:—MANSON, P. (1898). *Tropical Diseases.* 1st Ed., 447. London: Cassell & Co., Ltd.

Reference to an **Annual Report**: SWAZILAND (1937). *Annual Medical & Sanitary Report 1936*, p. 16.

Note.—The year of publication is not usually the year covered by the Report.

ABBREVIATIONS.

The abbreviations used are those shown in the **World List of Scientific Periodicals, 1934**, which conforms to the rules of the *International Code of Abbreviations for Titles of Periodicals*, Paris, 1930. In general, nouns have capital, adjectives small, initial letters; articles, conjunctions and prepositions are omitted; the place of imprint is added only when uncertainty might arise, e.g.:

Amer. J. Hyg.	*C. R. Acad. Sci., Paris.*	*J. Pharmacol.*
Ann. trop. Med. Parasit.	*C. R. Acad. Sci., Jekaterinoslaw*	*Ned. Tijdschr. Geneesk.*
Arch. Schiffs- u. Tropenhyg.	*Dtsch. med. Wschr.*	*Trans. R. Soc. trop. Med. Hyg.*
Bull. Soc. Path. exot.	*Indian med. Gaz.*	*Z. Hyg. InfektKr.*

The following contractions are in use, whether the number to be expressed is 1 or more (e.g. 1 c.c., 14μ, 45 kg.):—

centigramme, cg.	kilometre, km.	millimetre, mm.
centimetre, cm.	micron, μ.	ounce, oz.
cubic centimetre, c.c.	microgramme, mmg.	pound, lb.
cubic millimetre, c.mm.	milligramme, mg.	
kilogramme, kg.	millilitre, ml.	

In order to avoid dangerous error in dosage "grain" and "gramme" are printed in full.

REPRINTS AND BLOCKS.

Authors of Papers published in the TRANSACTIONS receive 25 reprints free of charge. Any additional number can be supplied on payment, provided application be made when submitting the Paper.

Blocks made for illustrations may be purchased by the author at half cost price.

NOTICE.—The attention of those who propose to contribute papers for publication is directed to "Editorial Notices" on page xx.

TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

VOLUME 43 No. 2

[illegible] 1949

CONTENTS

[The previous number of these Transactions, Vol 43 No 1 was published on 27th July, 1949]

TRANSACTIONS
OF THE
ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

VOL 43 No 2 SEPTEMBER, 1949

THE FORTY-SECOND ANNUAL GENERAL MEETING

of the Society held at

Manson House, 26, Portland Place, London, W 1,

on

Thursday, 16th June, 1949, at 7 30 p m

THE PRESIDENT,

SIR PHILIP MANSON-BAHR, C M G , D S O , M D , F R C P ,

in the Chair, followed by

Professor H E SHORTT, C I E., M D , D SC , D T M & H , Col I M S (ret)

(the new President)

BUSINESS.

REPORT OF THE COUNCIL FOR THE YEAR ENDED 31ST MARCH, 1949

The Annual Report of the Council was presented by the **Hon Secretaries**

Dr C A Hoare proposed the adoption of the Annual Report, and **Dr A K Cosgrave** seconded the resolution, which was carried

The **Hon Treasurer (Dr J C Broom)** read his Report He said that the financial position of the Society had improved during the year The excess of income over expenditure was £522 During the year the rents from resident tenants had increased by £925 , in a full financial year the increase would amount to about £1,800

Receipts from letting the Lecture Theatre have fallen by £132, but the income from this source is bound to fluctuate Repairs cost £539, of which we hope to recover £54 as war damage The income from sales of the TRANSACTIONS increased by over £500, while the cost of publishing fell by £181

Dr C Bentley proposed and Professor H B Day seconded the adoption of the Report. The resolution was carried.

ELECTION OF THE AUDIT COMMITTEE.

Dr W E. COOKE, Dr C. A. HOARE and Dr P C C GARNHAM were re-elected.

ELECTION OF PRESIDENT TWO VICE PRESIDENTS AND TWENTY COUNCILLORS.

The President Sir Philip Manson-Bahr then announced the result of the Ballot as follows —

President

H. E. SHORTT C. I. E. M D. D.SC., Colonel I.M.S. (ret.) Professor

Vice-Presidents

*GEORGE MACDONALD, M D. D P.H., D.T M Professor
*SIR JOHN TAYLOR, C.I.E., D.S.O. M.D., LL.D., D P.H., Major-General, I.M.S. (ret.)

Councillors

A. R. D ADAMS, M.D F.R.C.P D T.M.
JOHN BENNETT M.D., F.R C.P T M., D.T M. & H., Brigadier (lat R.A.M C.).
J S K. BOYD O.B.E., M.D., D.P.H., D.T.M. & H., Brigadier (late R.A.M.C., ret.)
J C. BROOM, O.B.E., M.D.
P A. BUXTON C.M.G., M.R.C.S., L.R.C.P D.T.M. & H., F.R.S., Professor
C. C. CHESTERMAN O.B.E., M.D., M.R.C.P D.T M. & H.
T H. DAVEY O.B.E., M.D D T.M., Professor
N. HAMILTON FAIRLEY C.B.E., M.D. D.SC., F.R.C.P F.R.S., Professor
R. M. GORDON, O.B.E., M.D., F.R.C.P D P.H., D.T.M., Professor
CECIL J HACKETT M.D. M.R.C.P D.T M. & H
R. BRUNEL HAWES, C.M.G., M.B., B.S. F.R.C.P
E. H. VERE HODGE, C I.E., M.D F.R C.P Lieut.-Colonel I.M.S (ret.).
E. M. LOURIE, M B. B.S D.P.H., D T.M. & H.
Sir GEORGE R. M ROBERT C.I.E M.D., F.R C.P D.T M. & H., Colonel I.M.S.
B. G MAEGRAITH, M.B., B.S., D.PHIL. Professor
T C. MORTON, O B.E., M.D., F.R.C.P D.T.M. & H. Air Vice Marshal R.A.F
F MURGATROYD M.D F.R.C.P T M.
L. EVERARD NAPIER, C.I.E., F.R. P
ERIC D PRIDIE, C.M.G D.S.O. M.B., B.S.
CHARLES WILCOCKS, M D. M.R.C.P D.T.M. & H.

* New Nomination.

The President (Sir Philip Manson-Bahr) This is my swan-song, and in singing it I propose to give an account of my stewardship over the last 2 years which have just flitted away

It is satisfactory to be able to announce that this Society is in a flourishing condition. The numbe of Fellows is greater than ever before. We are gradually approaching the 2,000 mark and, to be precise, we now have 1,961 Fellows.

My period of office has been marred by two great losses. I would refer once more to that of Dr CHARLES MORLEY WENYON to whom we all owe so much. The good he d d in his life will live after him. In the death of an

norary Fellow, Professor R P Strong, the doyen of tropical medicine, e feel that we have lost an international helper He, too, was a lifelong friend nd admirer of Sir Patrick Manson

During these last 2 years we have continued to make progress in refur-bishing our House

An outstanding feature of our evenings, and one which has contributed to congeniality, has been the inauguration of dinners before the meetings They have been well attended and much appreciated Owing to the wise pre-vision of Miss Wenyon, we are now able to hold them in the flat next door That the dinners are attractive is shown by the attendance sometimes of the Fellows' wives and other ladies

I wish to draw your attention to the display in the Manson case of the gold medals and insignia which once belonged to Sir Patrick Manson These have now been presented to this Society in perpetuity, a gift in accordance with one of the last wishes of my wife and one which has been made with the willing consent of all the members of my family

It is with a sense of gratitude that we acknowledge the gift of $1,000 from our good friend and Fellow, Dr Florence Frost, whom we are pleased to welcome here tonight This sum has been given for the purpose of purchasing books for the Library in memory of an old friend

A year ago I visited the Edinburgh branch of this Society and was hospit-ably received An address was given on Scottish Pioneers, during which much local satisfaction was caused by the discovery that most were of Scottish origin, including our new President, Professor Shortt

Together with members of the Council, I have attended combined meetings with the Royal Society of Medicine on two occasions, both of which proved a success

The Transactions of the Society have continued to maintain a high standard, and this is hardly to be wondered at since we have been so fortunate as to procure the services of Sir William MacArthur as Editor-in-Chief, and he has now under him a panel of Fellows to advise on the selection of papers for publication I might mention that we have had the honour of publishing Shortt and Garnham's epoch-making work in the Transactions, and also, in all humility, I would refer to the publication by Baylis of a new helminth in man, from a case which came under my care

It is pleasant to refer to the prominent part which our Secretary—Brigadier Boyd—played at the International Congress in Washington a year ago, an to congratulate him on his immortalization by the creation of the bacteri group, *Boydia* This is also the appropriate place to thank him sincerely f his loyalty, sincerity and unfailing courtesy to myself in our close associati At the same time I would like to say publicly how pleased we are to see Profe Fairley back in his old place, restored to health My best thanks are also to Dr F Murgatroyd in lending a helping hand to the Society whe were deprived of Professor Fairley's services

As you have just heard we are particularly fortunate in having Dr J C. BROOM as Treasurer Then there are our three lady secretaries, who have made life so easy and this Society a very happy ship, and as such I am sure it will long continue. Miss WENYON's mantle has fallen upon Miss HOPPER's shoulders, and it fits.

Finally in handing over to my successor I am so happy that it is Professor SHORTT He is one for whom I have the most intense admiration, not only as a most industrious and successful research worker but also as a man and as a sportsman.

I can only wish him as enjoyable a period of office as I have had.

The retiring President then invested Professor H E. SHORTT with the Badge and Chain of office, and inducted him to the chair

Professor Shortt I have listened in some embarrassment to the remarks of Sir PHILIP MANSON-BAHR, but I can assure you sincerely that I approach my task as President of our Society in all humility This is not to be wondered at when I look back upon my predecessors in office. I see those giants in attainment Sir PATRICK MANSON and Sir RONALD ROSS, Sir DAVID BRUCE, Sir WILLIAM LEISHMAN and Sir ANDREW BALFOUR, Professor STEPHENS and Colonel S. P JAMES—all now passed on. Happily still with us, I see GEORGE CARMICHAEL LOW Sir LEONARD ROGERS, Sir HAROLD SCOTT and my old chief and scientific hero, Sir RICKARD CHRISTOPHERS, and others I might mention while only recently departed is that greatest of English protozoologists, Dr C. M WENYON Surely this is a goodly company enough to make any man humble.

So far I have not mentioned my immediate predecessor Sir PHILIP MANSON-BAHR. He is the bearer of the most honoured name in our Society and I am only paying tribute to truth when I say that he has added lustre even to that name. Wherever tropical medicine is taught or learned, the name of MANSON BAHR is a—I was going to say household—perhaps I should more appropriately say hospital word. As editor of the most widely used book on tropical medicine, his name is known throughout the tropical world, and I suppose there is hardly a country which does not contain at least some of the still living products of his clinical acumen and skill.

He has made his own contributions to original research in subjects such as filariasis and amoebic dysentery and its complications, to mention only two, but it is as a born teacher that he is best known t thousands of students in all parts of the world We all know the skill often allied to a boisterous humour with which he drives home a clinical point at the bedside I often wonder how he does it as on one occasion when I saw him demonstrating the use of the sigmoidoscope to a large company and h remarked Now I hope everything goes right if the patient was a duchess I know the light would fail" —it failed!—but came on again to allow the completion of the demonstration. Surely a masterpiece of technique!

But, to be serious again, I feel that our Society should be something more than a place to read and listen to papers on subjects in tropical medicine Our membership, which includes teachers, research workers and clinicians, probably from every university in Great Britain and Northern Ireland, from most, if not all, of the Dominions and Colonies, as well as many from foreign countries, is such as to constitute a weight of authoritative opinion on tropical medicine equalled nowhere else in the world

I feel that we should use this informed opinion and this undoubted prestige in educating the body politic—I put it no more specifically—and in acting as the most authoritative and well-informed body to give advice, wherever questions of policy arise in connection with teaching or research in tropical medicine and the applications of such in clinical medicine, in hygiene and in field problems of all kinds in the tropics

This would apply at present in connection with the National Health Service, where problems concerned with tropical medicine are somewhat special ones and where it is not clear how our obligations can be best discharged It would also apply in connection with medical and industrial developments in our colonies in the tropics where costly errors due to lack of adequate knowledge and preparation might be avoided It would apply to the constantly changing conditions of education in tropical medicine generally and in the most useful application of established knowledge in the world of the tropics In no other body I know of is there such an aggregation of individuals with practical knowledge and experience on almost any problem likely to arise within the summer isotherms of 60° F North and South of the Equator

I make no specific proposals at this time, but I commend these ideas to our medical legislators in the confident assurance that if acted on, the results will be of infinite service to all those who dwell in or have to visit the tropics

You have heard the names of the two Vice-Presidents appointed by election The in-coming President has the privilege of nominating a Vice-President, and I have much pleasure in nominating Professor BRIAN MAEGRAITH

Now, Ladies and Gentlemen, there is some further business to transact and I do not wish to take up more of your time, but I would like to assure you that in occupying the Presidential chair my aim will be, honestly and to the best of my ability, to serve the Society and thereby, with your assistance, help to fulfil our obligations to all the peoples of the tropical regions of our Commonwealth and Empire

THE CHALMERS MEDAL FOR 1949

The President (Professor Shortt) The Chalmers Medal for 1949 has been awarded most appropriately to JAMES HENDERSON SUTHERLAND GEAR He was born in South Africa, educated in Johannesburg, and is a distinguished student of the Medical School of the University of Witwatersrand He has taken many medical qualifications, including a B Sc , D P H and D T M & H , and the London Diploma of Bacteriology

He is on the staff of the South African Institute for Medical Research and is a lecturer on tropical medicine at Witwatersrand In addition, he has a record of distinguished war service from 1940 to 1945 during which time he showed great ability in organization. In 1942 he was seconded to the International Health Division of the Rockfeller Foundation for work on yellow fever and homologous serum jaundice.

His chief contribution to medical science has been made since his return in 1945 to the South African Institute for Medical Research especially with reference to virus and rickettsial diseases. In the field he has been instrumental in demonstrating the existence of louse-borne typhus in South Africa, in showing the presence of flea-borne typhus in Natal and in working out the role of various ticks in the spread of South African tick typhus and the transovarian transmission of rickettsiae in the arthropod. Finally he has demonstrated the practicability of producing rickettsial vaccine after intranasal infection in gerbils.

In other directions he has also done good work—in meningococcal meningitis, in trypanosomiasis, blackwater fever relapsing fever and onyalai. It will therefore, be seen that Dr GEAR has performed a great work for his country and has carved out for himself at such a comparatively early age, an almost unique position. He is therefore a most worthy recipient of the CHALMERS MEDAL for 1949

Dr E H Cluver Dr GEAR has asked me to receive the Chalmers Memorial Medal on his behalf He is indeed sorry that he was unable to be present himself to thank you and your Council very sincerely for the award of this very high honour He would also have liked to express his gratitude to the Fellows of this Royal Society for it was at these meetings that he was inspired to take a special interest in the diseases of Tropical Africa. He also wished to take this opportunity of thanking his teachers, many of whom are here tonight, who stimulated him to make what contributions he has in advancing our knowledge of tropical diseases of Southern Africa.

On reading the list of previous awards, we were surprised to find that he is the third South African to be awarded this medal. The others were Dr MAX THEILER, who received it in 1939 and Dr E. M LOURIE, who received it in 1945 However Dr GEAR is the first South African to be awarded this medal for investigations carried out in South Africa. We are especially gratified that work done in our far-away country should not only not pass unrecognized but should be deemed worthy of this highly coveted award, which I have much pleasure in accepting on behalf of Dr JAMES GEAR.

AMENDMENT TO LAWS

Sir JOHN TAYLOR proposed the acceptance of the amendments, and Sir HAROLD SCOTT seconded.

These have been circulated to all Fellows of the Society through the medium of the TRANSACTIONS, Vol 42, Nos. 4 5 6.

Transactions of the Royal Society of
Tropical Medicine and Hygiene
Vol 43 No 2 September, 1949

ORDINARY MEETING

of the Society held at

Manson House, 26, Portland Place, London, W 1,

on

Thursday, 16th June, 1949, at 7 30 p m

The President,

Professor H E Shortt, C I E, M D, D Sc, D T M & H, Col I M S (ret)
in the Chair

PAPER

THE CHAGAS' DISEASE OF URUGUAY

BY

RODOLFO V TALICE

It is a great honour for me to pay my first visit to this famous Royal Society of Tropical Medicine and Hygiene, of which I have been a Fellow since 1936, and to be able to show to a public so well qualified, my film illustrating the disease as it occurs in Uruguay Chagas' disease, or American trypanosomiasis, occurs elsewhere in South America, in Brazil, Paraguay, and probably in British Guiana But before showing the film I should like to give a short paper about the disease, which is a summary of my 12 years' work This work up till now has shown the frequency of the disease only in Uruguay, but it has stimulate research in all other countries of South America

The work accomplished at the Institute of Hygiene of Montevideo my supervision, between 1937 and 1949, allows me to state the fo essential facts

Epidemiology

(*a*) There are in Uruguay only two common species of triatomes. One *Triatoma rubrovaria*, is widespread, of wild habitat and is only of secondary epidemiological importance. The other *T. infestans* whose common name is "vinchuca," a word of Indian ("Aymará") origin, is strictly domiciliary. The endemic region of Chagas disease is the same as the geographical distribution of *T. infestans*. One zone of the country is free from disease: it is the region South-East, on the Atlantic coast. This fact constitutes a problem of biogeography which is very interesting indeed.

(*b*) The number of human cases confirmed is almost 400 and 3,000 probable ones were studied: the mortality is less than 10 per cent.

(*c*) The investigation by means of xenodiagnosis (by the vector insects) has shown that 8 per cent. of the children carry trypanosomes in their blood. Many of them do not present symptoms which can be found by clinical and electrocardiograph examination. The percentage of infection is certainly higher than the figure given suggests.

Clinical

Most patients show the disease in an acute form, beginning with oedema in both eyelids, but my researches lead me to believe that non-oedematous forms without an apparent primary lesion, are more common although more difficult to diagnose.

The prognosis depends on the age. The course of the illness is usually benign, except in the case of very young children or first infection in adults. There must be, to my way of thinking, a premunition stage in many persons exposed to infection almost every night.

A variable percentage of acute cases (fewer in Uruguay than in Argentina or Brazil) show, after a longer or shorter delay, cardiac localizations which are capable of causing death.

The symptomatology of these acute varieties, sometimes protein in form makes differential diagnosis difficult.

Laboratory

(*a*) The most convenient method of diagnosis is a thick film (as for malaria), provided that it is well made, well stained and studied by a competent observer. The modification proposed by my collaborator EMPCART has the obvious advantage of allowing the parasites to retain the typical trypanosome appearance.

(*b*) The xenodiagnostic method is of value in experienced hands but it is a slow procedure. The culture of *T. cruzi* is easy to obtain, but blood culture is of no practical value. Inoculation of mice or young dogs has a limited utility.

(*c*) The Guerreiro-Machado reaction (complement fixation test) seems the only advisable procedure in chronic cases, but its technique is unfortunately

not yet standardized The variation of types of *Trypanosoma cruzi* causing the infection in human beings, is a fact which must be stressed

Treatment

I have experienced the same disappointing results stated by all other authors The German product, Bayer 7602, and the 70-A, an arsenical compound of EAGLE, from the Johns Hopkins Hospital, saves lives of young children suffering from severe forms, but the blood is not quite freed of the trypanosomes

Prophylaxis

All the measures taken against the insects are secondary ones, because of their peculiar biology Modern insecticides were employed in two systematic tests on a great number of huts The gammexane has recently given us interesting results The principal step to adopt consists in substituting the old rural dwellings of Latin America for more modern and hygienic ones The problem of the Chagas' disease can be stated to be a social and economic problem, depending entirely upon the State and not on individuals

A last point to state is the so-called antagonistic action of *T cruzi* against cancer made known by the Russian workers I have inoculated four advanced cancer cases without any favourable results and without any effect on the cancer cells

The Film was then shown

DISCUSSION

The President (Professor H E Shortt) I think you will agree with me that we have listened to an excellent and very succinct lecture and at the same time I cannot remember when I have seen a film that I have enjoyed so much. The film would be a perfect teaching film in every way I wish we could get a copy for our own classes, and perhaps Dr TALICE can tell us whether we can? I cannot think of anything better for teaching our own students. In fact, I would not now dare to put Chagas disease on the examination paper We must therefore congratulate Dr TALICE, and his charming secretary on a very enjoyable contribution, and I am quite sure that someone would like to ask questions perhaps on the actual subject matter or on the beautiful photography

Does he always use the adult stage of the triatoma in diagnosis? Could he use the larval or nymphal stage equally well? Another point is how long does he leave the adult bugs before he examines them for traces of parasites? A third question Can he tell us something more recent about the other trypanosome which has been described from South America?

Sir Philip Manson-Bahr Excuse me rising on this occasion to congratulate Dr TALICE on his teaching film. Two nights ago I was at a medical society in London where a series of American teaching films, produced by the American Medical Association, were shown very much on these lines. I was very much impressed with the teaching value of those films, more especially when it was demonstrated to us how to make a medical film, and the care and organization that go into it. It was pointed out that at least a fortnight should be spent in thinking out the details of the film before proceeding any further and that you should plan it in the same way as the planners of other educational films do. You should get your pathologist, your epidemiologist, your protozoologist and your clinician to think out all the details and get them into ordered sequence. Having done this and gone over all the difficulties, you then prepare a detailed account in a précis containing many typewritten pages of location and times, in which you propose to do the film. You should then put yourself in the position of the students and get them to come and criticize all the various stages, to see if they are intelligible to their attitude of mind. You next proceed to take the film in sections first of all from the geographical point of view—the map of the country the scenery the people the houses, the mode of life animals and so on. Then you proceed to illustrate it with clinical examples For example heart disease was very carefully shown with various tests required to bring out various phenomena. This one happened to be angina pectoris, and there were a series of photographs of the heart in various stages, also of the circulatory supply to the heart and finally of the different therapeutic methods used for curing the condition. This was very well shown—the form like the drugs and the plants from which they were derived After you had sat down and listened to this for an hour you were presented with a composite picture

range, which last offers few opportunities for prolific anopheline breeding Though the smallest of the three zones, comprising only 18 per cent. of the area, it contains 77 per cent. of the total population. *Llanos*, 36 per cent. of the area with 20 per cent. of the population, lies behind the mountains and is intersected by a series of rivers along the banks of which there is a belt of jungle liable to flood, and the intervening open country offers ample opportunity for anopheline breeding in the form of pools, ponds and lagoons. It is in consequence the most malarious of the three zones. Guayana, 46 per cent. of the area and 3 per cent. of the population, lies to the south of the Orinoco and is also intersected by several rivers, many of which, however are of a high acidity which prevents breeding of *Anopheles darlingi*, with the result that much of the country is non-malarious.

Typical meteorological data for the three areas are set out in graphic form in Fig 2. Except in the highlands the temperature is consistently over 18 C. In all zones, the mean annual temperature varying between 23 and 28° C. according to altitude, the relative humidity is high throughout the year except in limited areas where it drops in the dry season, and even in them only exceptionally below 60 per cent. Rainfall increases from north to south from an average of 1 000 to 2,000 mm. (39 to 79 inches) and varies in seasonal distribution in different parts, in general occurring as a mid year wet season. When analysed according to the modification of Gill's climatic zones introduced by GABALDON (1948), the southern portion of Guayana falls into the equatorial zone and the remainder into the para-equatorial zone. In both the season of malaria transmission is determined by rainfall only

The population is mixed white (20 per cent.), negro (8 per cent.), Indian (7 per cent.), and people of mixed blood (65 per cent.), and has an age and sex distribution typical of a country with an increasing population illustrated graphically in Fig 3 Density is on the whole low varying from 16 per square kilometre in the Costa-Cordillera to 0 2 in Guayana where, however the people are distributed in pockets of greater local density It has increased from 2,548 425 t 3,971,213 in the last 30 years, the increases being roughly equal in the three zones, but in Llanos this has been due to immigration and not to natural increase, the population actually decreasing in the years 1910 to 1922 when immigration was at its lowest. The general death rate is 21 1 per 1 000 the birth-rate 36·1 the infant mortality rate 117 and amongst that part of the population for which there is reliable certification of the cause of death, malaria is one of the five principal causes.

A separate anti-malaria service was established in 1936 It has received increasing financial support since then, with which it has carried out extensive surveys and several large drainage projects which reduced *A albimanus* and *A. darlingi*, and consequently reduced malaria, in several large towns. The methods used were expensive and did not touch the rural population, but when DDT was introduced sufficient knowledge of the epidemiology of the disease had been accumulated to start work on a large scale

mountains. Both the main vectors carry the disease in the Costa-Cordillera, a part of the Mexican zoogeographical subregion while *A. darlingi* alone is responsible in Llanos and Guayana which are nearer the centre of the Brazilian subregion. Though *A. albimanus* is found in the eastern parts of the Llanos, occasionally extending its area of occupation it is apparently not at home and disappears again from invaded areas, discouraged by the lack of sunlit pools.

The distribution of the species characteristic of the different regions is set out in Table I.

TABLE I.

ANOPHELINE SPECIES WHICH DIFFERENTIATE THE THREE REGIONS (PERCENTAGE OF POPULATED CENTRES POSITIVE FOR EACH SPECIES).

Species.	Faunal elements.	Costa-Cordillera.	Llanos.	Guayana.
A. albimanus	Mexican	18.4	2.8	0.0
A. apicimacula		23.3	15.1	5.0
A. pseudopunctipennis	Neotropical	77.2	43.4	10.0
A. albitarsis	Brazilian	18.4	0.8	75.0
A. darlingi		33.3	87.9	70.0
A. pessoai		0	9.4	10.0

Malaria rarely occurs in the Costa-Cordillera at altitudes over 500 m; the few infections seen above that level are apparently carried by *A. pseudopunctipennis* and are always below 1,000 m. In Guayana it occurs between 500 and 1,000 m. on the plateau of the Gran Sabana, where *A. darlingi* is prevalent. It seems that the factor reducing the prevalence of the two vectors in the Costa-Cordillera is the absence of large valleys and plains.

A. albimanus is most prevalent in areas of low rainfall usually between 500 and 750 mm. (20 to 30 inches) and *A. darlingi* in wetter regions, usually with a rainfall of 1,250 to 1,500 mm. (49 to 59 inches) though the relationship may not be a causal one but merely incidental to a distribution determined by other factors. *A. albimanus* is a sun-loving species and *A. darlingi* less so, as is shown by the following data on the percentage of catches in different types of breeding places in Barcelona, Anzoategui given by COVA-GARCIA (1943).

	Shade.	Diffuse light or some shade.	Sunlight.
A. albimanus	0.9*	18.1	81.0
A. darlingi	76.9	40.1	22.0*

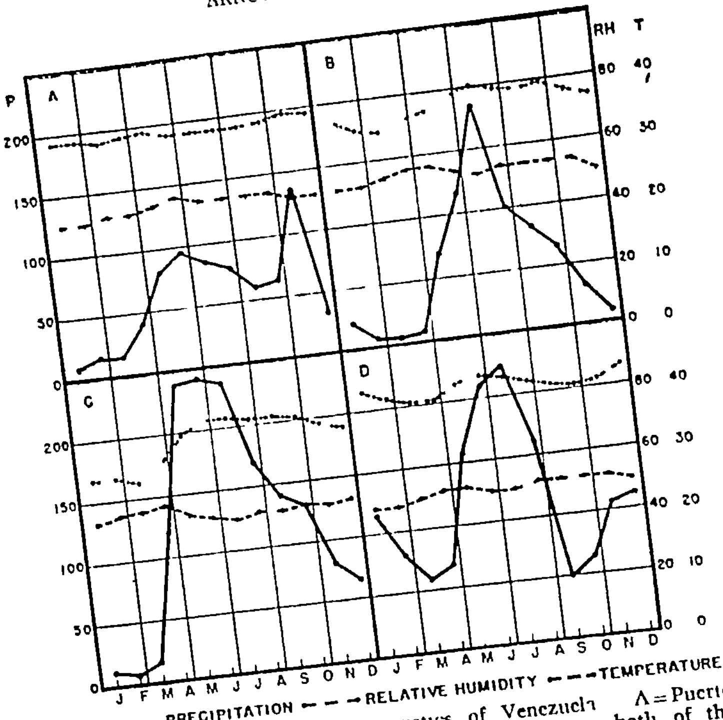

Fig. 2.—Meteorological characteristics of Venezuela. A = Puerto Cabello, Carabobo and B = Barcelona, Anzoátegui, both of the Costa Cordillera; C = San Carlos, Cojedes of the Llanos. D = Tumeremo, Bolívar of the Guayana.

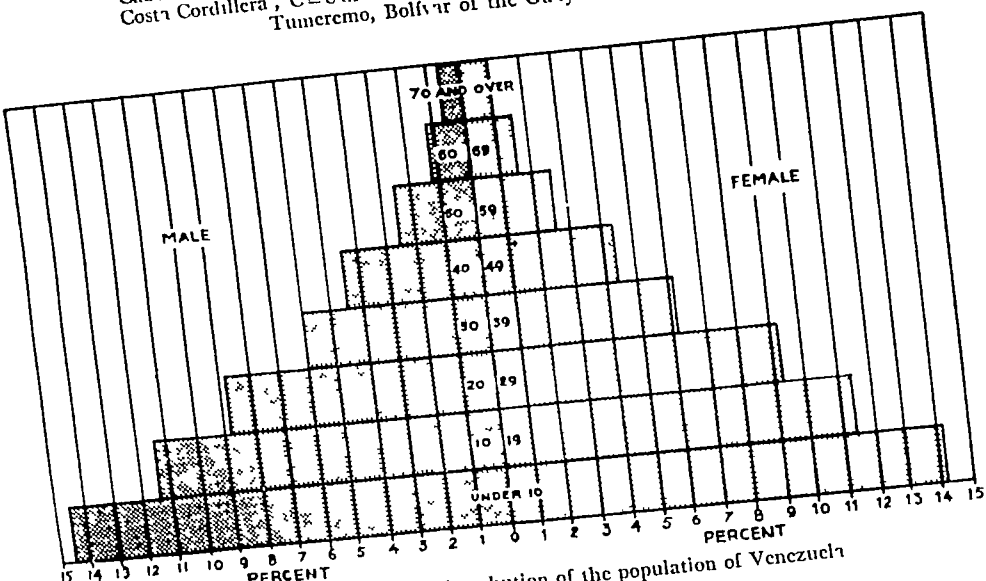

Fig. 3.—Age and sex distribution of the population of Venezuela.

Brackish marshes near the coast, with a salinity up to 1.7 per cent., are sources of *A. albimanus* but *A. darlingi* is banished by salinity from places near the coast. With reference to other types of breeding places, the indexes of preference from the same author and the same town show

	A. albimanus.	*A. darlingi.*
Rivers ...	0.8	31.0
Flowing streams	0.1	25.0
Overflowing pools	14.3	16.4
Ponds	16.8	6.0
Rain water pools	60	13.6
Others	8.1	7.3

This indicates that these vectors have a preference for different types of breeding places, and therefore in anti-malaria drainage when both species are present, it is necessary to take care of practically all the surface water.

A. darlingi is the most anthropophilic species, entering houses to bite humans in spite of animals being present in the neighbourhood. The animal baited stable traps used throughout the Caribbean to measure mosquito densities are useless in the control zones of *A. darlingi*. *A. albimanus* is more zoophilic.

TABLE II.

SEASONAL PREVALENCE OF *A. albimanus* AND *A. darlingi* IN BARCELONA, ANZOATEGUI.
(COMBINED FIGURES FOR 1941–1943.)

Month.	Capture stations (houses).	*A. albimanus.*		*A. darlingi.*	
		Number collected.	Density index.	Number collected.	Density index.
January	250	45	18.0	187	74.8
February	232	4	1.7	315	135.8
March	283	14	4.9	439	155.1
April	246	3	1.2	101	41.0
May	257	4	1.6	20	7.8
June	377	297	78.8	170	44.7
July	378	593	158.5	743	196.1
August	476	903	189.7	[illegible]	170.4
September	394	21	53.3	[illegible]	210.0
October	471	284	60.3	1,710	361.8
November	460	150	32.6	1,243	270.8
December	427	180	42	531	124.4

and its relation with the number of animals present may explain why it seems to be a much more efficient carrier in some countries than in others. *A. darlingi* is by far the better vector of the two, but as demonstrated by the writer (GABALDON, 1948) both are less efficient than the more potent of the Ethiopian and Oriental regions. The mean sporozoite index of *A. darlingi* is 0.9, and of *A. albimanus* 0.6.

The monthly prevalence of these vectors follows closely the monthly rainfall. It may be observed from this table that even in the dry season

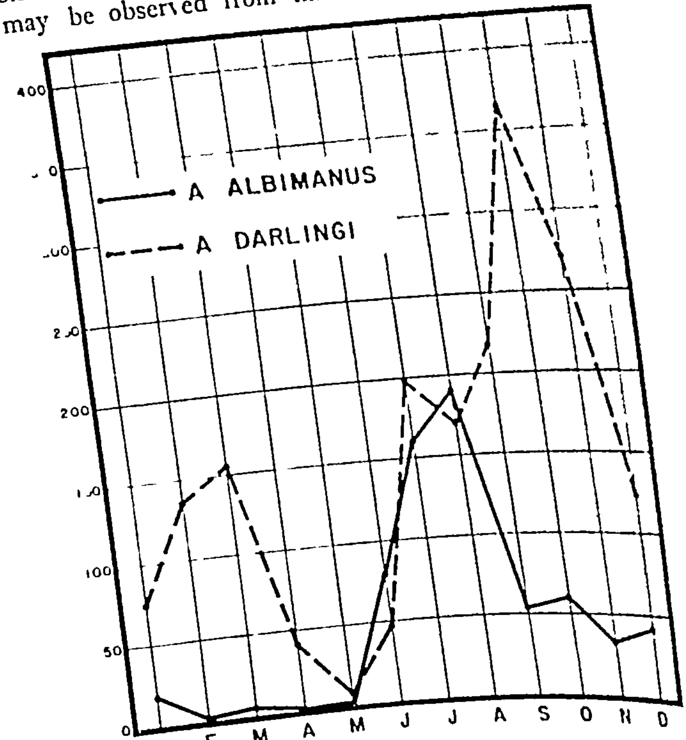

FIG. 4.—Seasonal periodicity of the adult-density indexes (houses) of *A. albimanus* and *A. darlingi* in Barcelona, Anzoátegui

some mosquitoes are found inside the houses. This means that in the para-equatorial climatic zone of malaria, where the mosquito cycle of the malaria parasite is interrupted only by lack of rainfall, even during the dry season there may be some transmission. This is important in relation to DDT work, as the insecticide has to be applied all the year around. This table, based on day-time captures, also shows that *A. albimanus* remains in houses during the day-time in Venezuela, which apparently is not the case in other countries.

Another interesting fact found in our studies is that some anophelines have non-annual cycles in the density of their population. In Table III, data

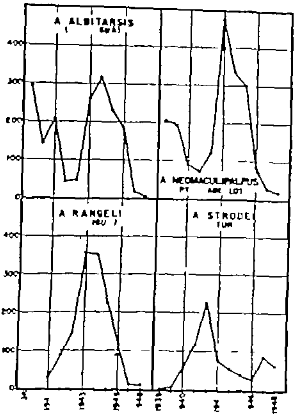

FIG. 5.—Non annual cycles in the larval density indexes of some Venezuelan anophelines.

for nine species are shown. This fluctuation in population density with a degree of periodicity has been described in other animals. In our case it is probably connected with changes in the food-cycle during the larval stage. It seems that this phenomenon has not been reported from other regions, probably for lack of standard indexes in entomological studies. These cycles are of special interest, as shown by the writer (GABALDON 1948), because they explain the similar cycles found in malaria prevalence. If this holds true in other zones, the whole epidemiology of malaria, in places where the anopheline cycles bring the density of the mosquito population below the critical point for transmission, may depend only on the food-cycle of the vector. Unfortunately the introduction of DDT will probably not allow investigations to elucidate this peculiar phenomenon with other vector species.

DISTRIBUTION

Malaria is less prevalent in the *Costa-Cordillera* than in the other two regions. *Three sectors of this region may be considered separately. The western sector* is formed by the valleys of Lake Maracaibo and of the Andean

Cordillera south and east of this lake. The northern portion of this sector is mostly occupied by *A. albimanus* in zones of low rainfall and to a smaller degree, by *A. darlingi* in jungle areas with higher rainfall. Here severe epidemics due to *A. albimanus* have been observed as a consequence of abnormally heavy rain. *A. darlingi* predominates in the central portion, but in the foothills of the southern portion, of the Lake Maracaibo or Orinoco Basins, there appears to be a problem due to *A. nunez tovari* and *A. pseudopunctipennis*. The known epidemics of the central portion are originated by the periodic fluctuations in range and density characteristic of *A. darlingi*. The spleen indexes of this portion are higher (sometimes even 100) than those of the northern or southern ones, an indication that *A. darlingi* is a more efficient vector.

The central sector of the Costa-Cordillera begins on the west with the Yaracuy Valley and ends in the east with the Tuy Valley and has the Andean Cordillera to the south. *A. albimanus* is present near the coast and *A. darlingi* in some inland places, but occasional infestation by this species occurs in the smaller coastal valleys and in the northern foothills. In the Cordillera is Lake Valencia at 450 m. with a large valley where both species are present. In the southern foot hills only *A. darlingi* prevails. The periodic fluctuations of this species are followed by severe epidemics in this sector. Islands of hyper endemic malaria with spleen indexes above 70 are common in the darlingi areas, but in the albimanus zone the indexes are below 50 generally under 25. Nevertheless, spleen indexes above 60 have been observed after increase of *A. albimanus* density due to the introduction of rice cultivation. In the smaller coastal valleys spontaneous disappearance of both species has been noticed. In *the eastern sector* formed by the States of Nueva Esparta and Sucre no malaria is present in the Cordillera the slope of the hills not allowing adequate accumulation of water for breeding places of the vectors. In Nueva Esparta, very little malaria has been found in the past as the very low rainfall of this island State does not allow *A. albimanus* to reach effective levels. In the State of Sucre, both vectors are present in the coastal valleys of the west, but in the eastern ones a problem due to *A. aquasalis* seems to be present.

The Llanos region is the area with highest malaria prevalence. However the incidence of the disease fluctuates according to the different zones. There is a large one near the Apure River on the south west, which is practically free of the disease the spleen indexes being below 10 per cent. Here *A. darlingi* the only vector of the region, is absent, probably because rivers without heavy forest and with marked fluctuations of level are not suitable to its maintenance during the dry season. Big epidemics have not been observed in the Llanos, where the endemicity of the disease is moderate in the southern portions, the spleen indexes being below 50. On the other hand, hyperendemic malaria is found with some indexes of 100 in the northern parts.

The Guayana, the largest of the three regions, is mostly covered with a dense tropical forest, small sectors of open country occurring on its northern

limits, and on its southern side there is a large rolling plateau covered by savanna. *A. darlingi* is the only vector but here the population is less rural than in the Llanos, a factor which is probably responsible for the lower levels reached by malaria in this zone, where the spleen indexes are generally below 50, although one of 85 was found. In some areas of the open country of the north-east the vector is absent and the spleen indexes are about 5. In the south-west large rivers (Atabapo, Guainia) with acid black waters have on their banks villages free of malaria, as no suitable breeding places of *A. darlingi* are present.

"Condition" of Malaria

Malaria prevalence has been divided by the writer (Gabaldon, 1949) into two types of different practical significance: basal malaria, the consequence of vectors developing in natural breeding places, and additional malaria, the result of vectors having their aquatic cycle in artificially produced breeding places. In Venezuela we have to deal mostly with basal malaria, and it is against it that our main efforts have been directed. Additional malaria is found in *A. albimanus* territory mainly as a consequence of rice cultivation and road construction, and of this latter factor in *A. darlingi* areas.

TABLE IX

RATIOS OF ENDEMICITY AND OF EPIDEMICITY IN FOUR VILLAGES OF THE STATE OF CARABOBO BASED ON SPLEEN INDEXES OF CONSECUTIVE YEARS (FROM GABALDON, 1949)

Pueblo	Spleen indexes					Ratio of	
	1st year	2nd year	3rd year	4th year	5th year	Endemicity	Epidemicity
Naguanagua	2.6	4.8	46.2	28.2	6.[illegible]	1	[illegible]
Güigüe	3.5	[illegible].1	37.2	1.[illegible]3	0.0	1	7
Urama	70.2	92.8	68.4	76.0	70.6	14	1
Morón	75.3	98.6	85.[illegible]	87.8	72.7	1[illegible]	1

Another aspect of the epidemiology of the disease which it is important to understand before the beginning of a nation-wide malaria control programme, is the "condition of malaria" in the country. The ordinary surveys based on the spleen and/or parasite indexes give an idea of the "situation of malaria," that is, the prevalence of the disease in a community at the moment of examination, or immediately before. The "condition of malaria" means the tendency of this disease to change its prevalence from year to year in a given area, changes measured by annual variations in the spleen rate independent of seasonal changes. The measures used are (1) the ratio of endemicity, or the lowest spleen rate observed in a 5-year period divided by 5; and (2) the rates of epidemicity, or the highest spleen rate observed in such a period divided

by the lowest. As seen in Table IV when the ratio of endemicity is low spontaneous reduction of malaria occurs during some years in a region, and a decrease of the disease obtained after a control project may not be significant On the other hand, when the ratio of endemicity is high, a decline of malaria would undoubtedly be the result of the measures used. The extensive work done in Venezuela before the introduction of DDT which allowed the classification of areas with low and high ratios of endemicity has permitted the development of the nation-wide campaign with this insecticide on a sound basis.

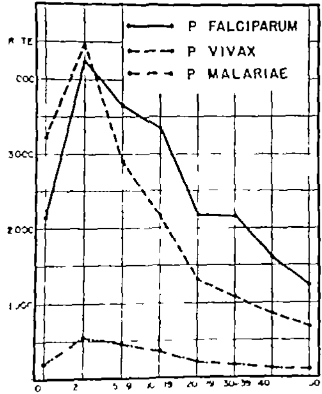

Fig. 6.—Age distribution of the three malaria parasites based on the morbidity rates presented in Table VI

Another important fact shown by the study of spleen indexes is that in spite of different degrees of malaria prevalence as indicated by the height of these indexes, the age group 10 to 14 years shows no more splenomegaly than the group of 5 to 9 years of age (Table V). This finding demonstrates that even our endemic malaria is of a lesser degree than that of the Ethiopian and Oriental regions, which is related to the lesser efficiency of our anopheline vectors and was a reason for the adoption by the Pan American Malaria Commission (1947) of the age group 5 to 14 as the standard group for spleen indexes in this region.

P falciparum is the predominant species of parasite in Venezuela, a common finding near the Caribbean. The species decreases from here southwards in the Neotropical region, as was shown by the writer (GABALDON, 1948) The age prevalence of parasites (Table VI) indicates that the highest incidence is found in the ages 2 to 4, probably another proof of the lower endemicity of the region Furthermore, it may be also observed that below 5 years *P vivax* is more prevalent than *P falciparum*, contrary to what is seen from 5 years on, apparently a sign of the stronger immunity produced by the benign tertian parasite This age distribution of morbidity is not correlated with the age distribution of mortality (Table VII), where the highest is found in the groups

TABLE V

AGE DISTRIBUTION OF SPLENOMEGALY IN LOCALITIES OF DIFFERENT SPLEEN INDEXES (AFTER GABALDON AND GOMEZ MARCANO, 1948)

Spleen indexes	5 to 9 years of age			10 to 14 years of age		
	Number examined	Enlarged spleen	Percentage	Number examined	Enlarged spleen	Percentage
0– 4	8,823	249	2 8	11,297	344	3 0
5– 9	7,758	674	8 7	10,535	940	8 9
10–24	14,053	2,336	16 6	19 032	3,223	16 9
24–49	6,448	2 090	32 6	8,601	3,030	35 2
50+	3,594	2,263	63 0	4,184	2,667	63 7
Total	40,676	7,620	18 7	53,649	10,204	19 0

TABLE VI

MEAN ANNUAL MORBIDITY RATES PER 100,000 FOR EACH SPECIES OF PARASITE BY AGE-GROUPS (DATA FROM 1938–1945 IN FIVE TOWNS ACARIGUA, PORTUGUESA, BARCELONA, ANZOATEGUI, MATURIN, MONAGAS, PUERTO CABELLO, CARABOBO AND SAN CARLOS, COJEDES)

Age-groups	*P falciparum* Rate	*P vivax* Rate	*P malariae* Rate
0– 1	2,153	3,237	204
2– 4	4,257	4,471	537
5– 9	3,656	2,912	453
10–19	3 359	2,195	351
20–29	2,184	1,317	216
30–39	2,166	1 076	175
40–49	1,616	854	126
50+	1,234	675	112

TABLE VII.

MALARIA MORBIDITY AND MORTALITY RATES PER 100 000 INHABITANTS BY AGE AND SEX IN VENEZUELA.

Morbidity rates.			Mortality rates.		
Age-groups.	Male	Female.	Age-groups.	Male.	Female.
0– 1	8,175	7 173	*Under 1*	572	570
2– 4	12,938	11,604	1– 4	343	320
5– 9	9 029	8,917	5– 9	129	110
10–19	7,222	8 035	10–19	64	64
20–29	6 790	4,049	20–29	84	84
30–39	4 490	4,340	30–39	136	85
40–49	3 371	3,484	40–49	165	112
50 +	2,548	2,948	50–59	168	130
			60–69	237	176
			70 +	395	270

The morbidity rates are based on figures from Acarigua, Portuguesa; Barcelona, Anzoátegui Maturín, Monagas Puerto Cabello, Carabobo and San Carlos, Cojedes; for the period 1941 1945 The mortality rates are for Venezuela in 1942, the year with highest malaria in the period 1941 1945.

below 1 year and above 50 years. In the same table it may be observed that the rates are in general higher for males than for the females. This is especially so in the group below 5 years of age, a finding which has always been observed when large numbers have been studied. This may indicate a greater susceptibility of males or may only be a consequence of the general propensity of the male to contract disease and die at the early years.

TREND LINE.

The malaria mortality rates have been decreasing in the three regions since 1916–1920 when the highest were observed. A study of the trend of this decline made by the method of the least squares, indicates that the largest drop has been in the Llanos, the values for the three regions being

	Y = a + b X
Costa-Cordillera	= 280 + (− 6X)
Llanos	= 668 (− 14X)
Guayana	= 199 + (− 2X)

Fig 7 shows the trend lines for the three regions based on data published by GABALDON and DE PÉREZ (1946) for the period 1910–1945 that is before the introduction of DDT

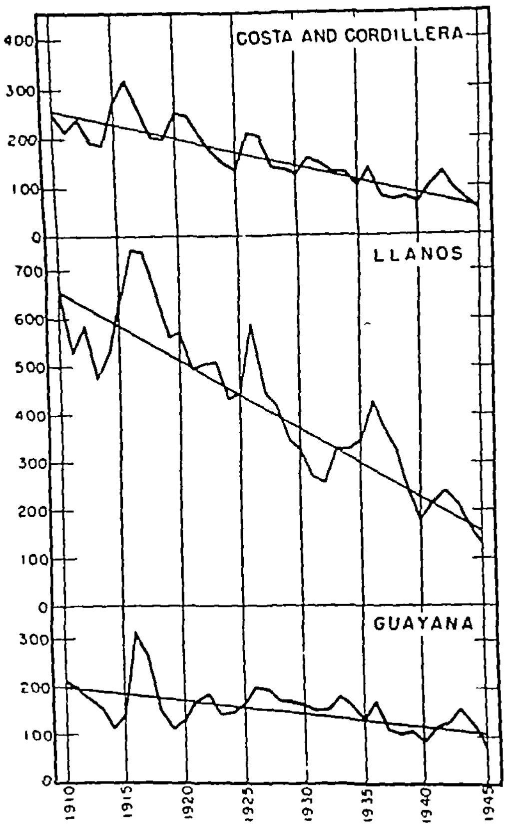

FIG 7—Trend line and non annual cycles of malaria mortality rates in the three zones

This long-term spontaneous reduction of malaria in Venezuela is hard to explain, though it may only be the decline of a periodic wave, which reached its peak in 1916 to 1920 It seems that during the last century malaria was invading some regions with higher intensity, but there are no available data to demonstrate this fact On the other hand, a study of the evolution of foreign commerce and Government expenditure indicates that there has been a conspicuous improvement in the general economic conditions of the country since 1921 to 1925, which should be reflected in the standard of living of the people, and more anti-malaria drugs and insecticides have been used as shown by import statistics This may be an alternative explanation But the decrease of malaria which has been observed, marked as it is, does not account for the sudden drop that the disease has shown after the introduction of DDT on a nation-wide scale

MALARIA PERIODICITIES

In Venezuela two types of malaria periodicities have been observed, the annual seasonal cycle and the non-annual 5-year cycle A careful consideration of Fig 7 shows that malaria death-rates have not dropped continuously, but have declined by waves which have a period of about 5 years, particularly in the Costa-Cordillera, where they have been more regular than in other areas

A careful statistical study of this phenomenon, based on the data published by GABALDON and DE PÉREZ (1946), shows some interesting facts, as in the following examples for the State of Carabobo, one with most typical figures First, the difference between the observed malaria death-rate for each year and the theoretical malaria death-rate for the same year, as given by the value of the trend-line for that year was taken This difference was divided by the standard error of the arithmetic mean of the series formed by the malaria death-rates for the period 1910 to 1945 These values of x/σ, plus or minus 0,

were plotted on line A of Fig 8. It may be observed that most of the cyclical increases are above 3, which means that they are statistically significant, and therefore that a typical 5-year periodicity of malaria does exist. Similar procedure was followed for the general death-rate plotted on line B and for the general death rate minus the malaria death rate plotted on line C. Now it may be seen that each significant increase of the curve on line A is accompanied by similar increases of the curves B and C. This indicates that when malaria mortality increases, the general death-rate and the death-rate due to other

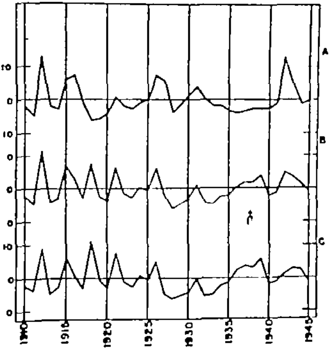

FIG 8—Five-year periodicity of malaria in Venezuela as shown by death rates from the State of Carabobo

diseases also increased. A closer observation of the curves shows that only in 1918 was there an increase in the general death-rate that was not accompanied by an increase of the malaria death-rate This exception was due to the severe epidemic of influenza, which affected also other countries of the world. But the height reached by the general death-rate in this year is lower than the one observed in 1915 when there was the most severe epidemic of malaria found in our records.

These 5-year cycles are also reflected in the spleen indexes of epidemic zones. They are apparently the consequence of similar periodic cycles of population and range fluctuation of *A darlingi* as was mentioned above. *A*

albimanus may also play a role, but probably in a minor degree Careful consideration should be given to these cycles in the evaluation of malaria reduction by control programmes

The annual seasonal periodicity of malaria in Venezuela also has typical features There is only one peak during the year in regions with one peak in the rainfall The lowest point of the curve is found in the months of March, April, or May, at the end of the dry season The highest and the lowest points of the curve are correlated with the ratio of endemicity of the place The

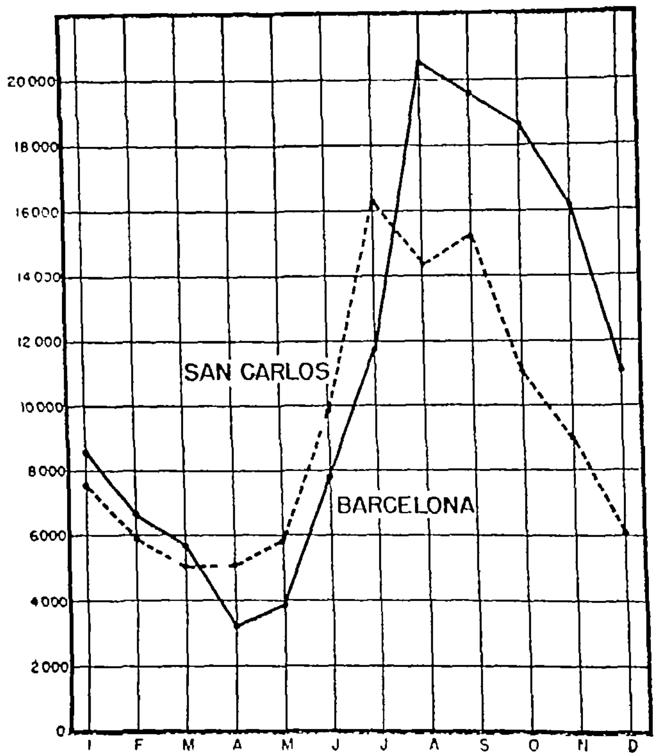

FIG 9—Seasonal periodicity of the malaria morbidity rates per 100 000 (microscopical diagnosis) in Barcelona, Anzoátegui and San Carlos, Cojedes

peak of the curve in Barcelona, Anzoategui (20,533 in August) with a ratio of endemicity of 2, is much higher than in San Carlos, Cojedes (16,274 in July), with a ratio of endemicity of 6 (Table VIII), and the lowest point, on the contrary, is higher in the last town (5,069 in March), than in the first one (3,222 in April) The ratio of amplitude (20,533/3,222 = 6 4 for Barcelona, and 16,274/5,069 = 3 2 for San Carlos), measures the epidemic trend of the seasonal wave, and confirms the values of the ratio of endemicity

The behaviour of each parasite species in the seasonal wave is different.

TABLE VIII.

MONTHLY MALARIA MORBIDITY RATE PER 100 000 IN THE PERIOD 1941 1945 TO SHOW SEASONAL PERIODICITY

Month.	Barcelona, Anzoátegui.		San Carlos, Cojedes.	
	Positive slides.	Rate.	Positive slides.	Rate.
January	846	8 637	[illegible]	7 677
February	[illegible]	6 66[illegible]	[illegible]	[illegible]
March	361	6 711	190	[illegible]
April ...	197	2,222	184	5 075
May	46	2,601	[illegible]	[illegible]
June ...	470	657	256	9 120
July ...	745	11 785	610	16,274
August	1,293	[illegible] 533	535	14,333
September	1 107	10 877	[illegible]	18,226
October	1 177	10 619	414	11 095
November	899	10 101	236	8 993
December	699	11 057	[illegible]	6 039
Total ...	9,308	11 137	4 106	9,593

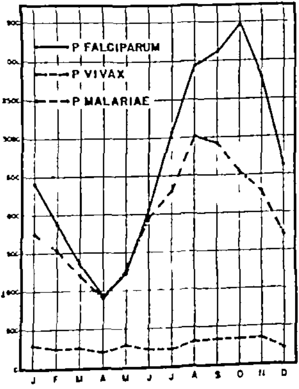

FIG. 10.—Seasonal periodic of the three malaria parasites based on the morbidity rates presented in Table IX.

P falciparum behaves as in other climatic zones of malaria (*a*) the decline during the season of reduced transmission is steeper than with the other parasites, (*b*) the rise is slower than in *P vivax*, (*c*) the peak is reached later than in *P vivax*, (*d*) the ratio of amplitude (4,468/933 = 4 8) is higher than in *P vivax* (3,013/892 = 3 4), and in *P malariae* (348/206 = 1 7)

P vivax behaves in a slightly different way than in other climatic zones of malaria (*a*) during the reason of reduced transmission there is no clearly marked relapse wave, (*b*) during the same season in some places the rates are higher than for *P falciparum*, this being especially so if the numbers of children are proportionately large, probably because of the higher prevalence of *P vivax* in the early years, and (*c*) the peak of the wave in the active transmission season is lower than in *P falciparum*

P malariae, on the other hand, shows (*a*) a definite relapse wave during the season of reduced transmission, (*b*) a longer flat or irregular peak during the season of active transmission, (*c*) lower rates at all times The descending limb of the transmission season curve is always longer than the ascending limb for the three parasites, as has been found in other zones (See Table IX)

It must be emphasized that this seasonal periodicity of malaria, typical of the para-equatorial zone, has no seasons of interrupted transmission This has been clearly shown when DDT spraying completely intercepted transmission as in Barcelona, Anzoategui, and other places (Fig 14) In these cases,

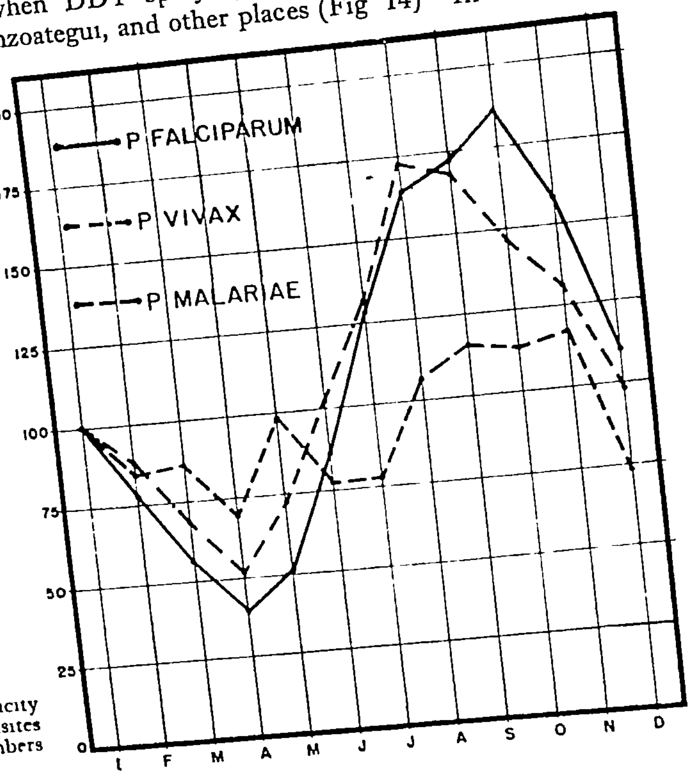

Fig 11—Seasonal periodicity of the three malaria parasites based on the index numbers of Table IX

after spraying at the beginning of the dry season, the observed rates for that season were lower than in all the former dry seasons, which clearly shows that transmission had been only reduced in former years. Therefore, as some transmission goes on during the dry season it is possible that the relapse wave of *P vivax* is obscured by new infections, though the fact that no relapse wave was observed after DDT spraying probably means that the *P vivax* strains present here do not produce as many relapses as in other countries.

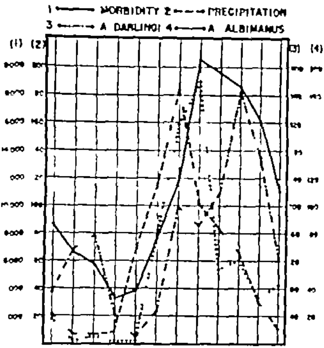

Fig. 12.—Correlation between malaria morbidity rates and rainfall and adult density indexes in Barcelona, Anzoátegui.

TABLE IX.

MONTHLY MORBIDITY RATES PER 100,000 FOR THE THREE SPECIES OF MALARIA PARASITES (BASED ON DATA FOR 1939–1945 FROM ACARIGUA, PORTUGUESA; BARCELONA, ANZOÁTEGUI; MATURÍN, MONAGAS; PUERTO CABELLO, CARABOBO; AND SAN CARLOS, COJEDES).

Month.	*P falciparum.*		*P vivax.*		*P malariae*	
	Rate	Index.	Rate.	Index.	Rate	Index.
January	[illegible],400	100.0	1 744	100.0	[illegible]	100.0
February	1 860	77.3	1 639	87.7	231	84
March	1,346	56.1	1 193	68.4	257	95.3
April	933	38.9	69	51.1	[illegible]	68.1
May	1,[illegible]	30.9	1 [illegible]01	7[illegible].3	[illegible]	95.3
June	[illegible],007	83.7	1 949	111.7	[illegible]	78.8
July	3 071	127.9	2,290	131.0	231	77.5
August	3 933	163.9	3 013	17	317	104.4
September	4 123	171	[illegible].4	167.7	344	113.4
October	4 46	169	2 453	140.4	[illegible]	112.4
November	3 7	157.6	[illegible]	131.0	31	114
December	636	109.6	1 703	97.6	31	73.2

naturally came to be the responsibility of the section of anti-malaria engineering of the Servicio de Fomento Antimalárico and we owe much to Dr GERARDO GONZALEZ and Dr SALVADOR CARILLO, the chiefs of the service, and Dr ARTURO LUIS BERTI the chief of the section. The headquarters is at Maracay and has attached to it a chemical laboratory and a research laboratory under Dr JOHN MAIER.

The field services are in the hands of zone engineers of the Division de Malariologia in different States or in their absence, in the hands of the zone doctors in charge of the regional services of epidemiology and medical activities. In each zone there is also a DDT inspector who supervises the work of DDT squads does survey and checking work, and generally helps his chief

DDT squads consist of six to eight uniformed sprayers with a leader and auxiliary staff such as drivers. They are organized in different ways according to the communications of the district to work from trucks, motor boats, wheel barrows, trolleys, horses or on foot. The truck squad is the basic one in future it will be mounted on small vehicles of the weapon carrier or jeep type though larger vehicles have been used in the past. These vehicles carried, as well as the men, a supply of DDT two 400-litre tanks for suspensions, and one or two 200-litre tanks for solutions, carrying sufficient supplies to work away from its base for about a week. With smaller vehicles some modification of this is needed, tanks for suspensions are eliminated, the suspension being prepared at the time of use. When trucks cannot be used appropriate squads are organized around the other forms of transport mentioned

The Division has an operations manual with a section devoted to DDT work. It is a loose-leaf stencilled book, giving detailed working instructions which is constantly kept up to date some parts having gone through three editions. This manual is an essential part of the work, which has now been copied elsewhere. It includes instructions on other types of skilled work involved, such as vehicle maintenance

On April 1st, 1949 the staff included 1,581 persons classified as follows 19 physicians 9 engineers 2 entomologists 2 chemists 13 administrators 1 meteorologist 36 malaria inspectors 17 topographers 11 DDT inspectors 98 urban and rural visitors 135 draftsmen photographers, clerks and laboratory technicians 59 squad leaders 373 sprayers 106 drivers 5 pilots 49 other skilled workers and 626 unskilled workers.

The Division uses only technical grade DDT conforming to the U S A. Joint Army and Navy specification D-58-A, and 50 per cent. wettable powder for which a special specification has been found necessary t avoid inefficiency due to the use of rap dly settling and otherwise unsatisfactory products.

This specification is (*a*) particles not larger than 40μ i size (*b*) complet wetting f 2 5 grammes in 50 ml. of water in less than 2 minutes and (*c*) not less than 2 per cent. of DDT in the middle of the column of a 2·5 per cent. suspension placed for half an hour 100 ml. glass-stoppered cylinders.

A 5 per cent solution in kerosene is used only in painted houses, and is prepared *in situ* in the tanks on the trucks with locally purchased kerosene which is everywhere readily available. Suspensions are used elsewhere, that is, in most houses, and they have been shown to be more active under our conditions for longer periods than solutions or emulsions (MAIER, RENDTORFF and SUAREZ, 1948). Samples of all insecticides are assayed chemically before purchase under the supervision of an officer of the Pan-American Sanitary Bureau, and the chemical laboratory has proved to be an essential part of the organization if a high standard is to be attained.

Several kinds of sprayers have been used; in our experience the best is that designed by TRAPIDO (1948). The best nozzles are those which throw a fan-shaped spray of 900 or 1,800 ml. per minute at 60 lbs. per square inch pressure, though the smaller ones clog except when used on Trapido's pump. The strength of solution or suspension is varied with the nozzle used and the quantity of fluid applied. It is this nozzle size more than anything else which modifies the dose applied, and as the aperture of the nozzle increases with use the tips are changed at least once a month. Using the small nozzle a 5 per cent suspension sprayed so as to treat 20 to 25 square metres per minute the dose applied is 200 mg. DDT per square foot (2 grammes per square metre).

All houses in malarious areas are treated except that the non-malarious core of large towns may be omitted. As a preliminary, houses, roads and distances are studied and a detailed plan of action given to the squad leader, who deputes a man to go a day ahead of the team to prepare the population, give advice on such matters as the protection of food, and make essential notes. On arrival of the squad, the leader checks and supervises work, keeps his records, assigns the driver to the business of preparing suspension and filling pumps and to each of the others a group of houses for treatment. The whole interior of all houses, stables, latrines and other shelters is treated, including verandahs, eaves and the under surface of furniture. Work is inspected both regularly and without notice, by the zone officer and his inspector; entomological and parasitological data are collected by another organization, and reports on these examinations, together with the work reports of the squad, serve as a basis for controlling the quality of work from the headquarters.

During 1946 spraying was repeated every 3 months, in 1947 and 1948 every 4 months, and in 1949 every 6 months. In 1946 and 1947 the dose given was 100 mg. per square foot, but this was doubled at the end of 1948, as experience has now shown that 200 mg. per square foot every 6 months is adequate, and the most economical cycle for our needs. It is now probably fixed because the removal of DDT as by the cleaning of walls makes it undesirable to increase the time interval. Work is continued throughout the entire year because transmission never really ends and social legislation makes it very undesirable to employ labour on a seasonal basis.

In the fiscal year 1945–1946, when the DDT campaign was started, there was no special provision for this activity in the budget of the Division de Malario-

logu. The money spent was taken from the general budget of the Section of Anti-malaria Engineering From that time on, amounts for DDT work have been included as follows

Fiscal year	Division budget. Bs.	DDT campaign. Bs.	Percentage.
1946–1947	8 143 420	1,850 000	22 7
1947–1948	10,537,600	4 000 000	38 0
1948–1949	12,652,424	5 600 000	44 2

From these figures may be observed the increasing role DDT has played in our work. Also it is worth noting that the total budget of the Division, which has been enlarged continuously represents an expenditure per capita of Bs 2 81 (U S. $0·84) in 1948–1949 which probably is one of the largest devoted to malaria control work by any tropical public health administration. This is a clear sign of the importance that Venezuela has attached to this disease which was one of the worst scourges of the country and of the sound basis on which public health work is conducted in this Republic.

At present (June 1949) there are 60 DDT squads in Venezuela, 43 of them with motor transport, eight on foot with wheel-barrows, four conveyed by motor boat, four by horses, and one by trolley and the trend is to increase the number of the non motor transported squads to reach the more inaccessible places.

Table X shows the progress of work in 3 years, during which work has increased tenfold until, in 1948, the number of houses treated was 168,472 and the number of persons directly protected reached 863 498. There are about 200 000 houses with over a million inhabitants in the treated areas, but some houses were excluded because they are in the centres of towns, and others because the occupants were away or for one reason or another refused treatment The relatively small rise in the percentage of total costs represented by DDT is due to a number of factors, notably the more economical spraying cycles latterly adopted and the increasing skill and efficiency of the staff It is still below most of those given by PAMPANA (1948) in spite of the relatively high cost of labour in Venezuela and the difficulties of communications. The total costs are rising and may be expected to rise still further because work which was originally started in the more accessible and densely populated areas is steadily being extended to more difficult, and more costly areas. The present intention is to spray all of those sparsely populated or mildly malarious pockets which have been left in the areas due to be treated in a last difficult and expensive effort to see if malaria can be entirely eradicated from large tracts of country Finally though the borders of the territory may remain infected by carriers from untreated places, the total number of houses needing spraying may be much reduced.

TABLE X.

PROGRESS OF THE SPRAYING PROGRAMME

	1946	1947	1948
Localities sprayed	272	1,251	2,498
Average number of houses in each	64	66	67
Sprayings per year	1 7	1 9	2 2
Number of houses protected	17,311	82 388	168,472
„ house sprayings	28,905	156,997	372,160
„ persons protected	89,055	414,538	863,498
DDT used (kg)	7,791	51,779	165,999
Grammes DDT per spraying *per capita*	55	66	88
Cost of DDT as percentage of total	18 0	22 9	28 6
Cost per inhabitant (Bolivars)	2 12	2 18	2 18
Houses to be sprayed	433,878	443,238	452,615
Percentage of houses sprayed	4 0	18 6	37 2

RESULTS

Detailed information on our experience in the nation-wide campaign against malaria in Venezuela is presented elsewhere (GABALDON, GONZALEZ and BERTI, 1947 , GABALDON, BERTI and GONZALEZ, 1948 , GABALDON, BERTI and CARRILLO, 1949), but, unfortunately, due to printing difficulties, these papers are still to be published A summary of the results so far obtained is presented here It should be repeated, in order to understand properly the successful progress obtained in the campaign against malaria in Venezuela, that within the malaria zone the worst infected areas have been protected with DDT Inside these areas some pockets have been left without DDT due to their inaccessibility, but because of this very factor, these pockets have little influence on the data showing the results attained At the beginning it may be said that where DDT has been sprayed a reduction of malaria has been observed Therefore, it will be seen, that *A albimanus* or *A darlingi* transmitted malaria is checked by DDT

To present a large number of examples in reference to the malariometric indexes or other data demonstrating the effect of DDT in Venezuela is not possible in this summary for lack of space But figures taken from the more typical places will give an idea of what has been obtained

EFFECTS ON THE MALARIA INDEXES AND RATES

Every year during the dry season the zone doctors have made spleen indexes of some towns and pueblos of their territory This work has amounted to the examination each year of 20,000 to 25,000 school children of both sexes, between the ages of 5 and 14 Except for schistosomiasis, there is no other endemic disease prevalent in Venezuela producing splenomegaly As schisto-

FIG. 13 ( )—Progress of the DDT-spraying programme in Venezuela

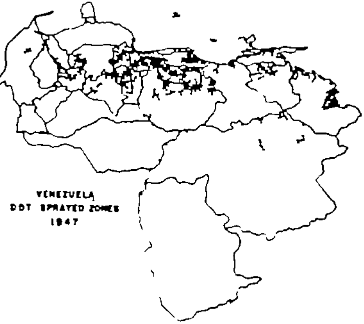

FIG. 13 (b)

somiasis is present only in certain limited areas, most of them non-malarious, it does not interfere in practice with malaria splenomegaly. Slides were taken only from children found with splenomegaly, and therefore no parasite indexes were obtained. The children were examined lying on their backs with flexed legs and bare abdomen. Boyd's scale was used and the average enlarged spleen was calculated with the writer's (GABALDON, 1945) modification. The racial element was not taken into consideration in these indexes, because most of the children examined were mestizos or white, the Amerindian and Negro races being represented in too small numbers to be considered separately. Furthermore, there are not significant differences in racial habits and diet, all the people living in the same communities without any racial segregation.

A study of the effect of DDT (Table XI) has shown that attention should be paid to the ratios of endemicity and epidemicity. Guacara was not sprayed but its spleen index came down following the 5 year cycle of malaria. Guigue, with identical ratios of endemicity and epidemicity to Guacara, had already reached a spleen index below 5 per cent when it was sprayed. Patanemo with similar ratio of endemicity and epidemicity, showed a spleen index which reached the normal of under 5 per cent in 2 years after the first spraying. On the other hand, Moron and Urama, with a very high ratio of endemicity and a very low ratio of epidemicity, just the contrary of the other two pueblos, had spleen indexes above 10 per cent after 3 years of spraying. But this divergence

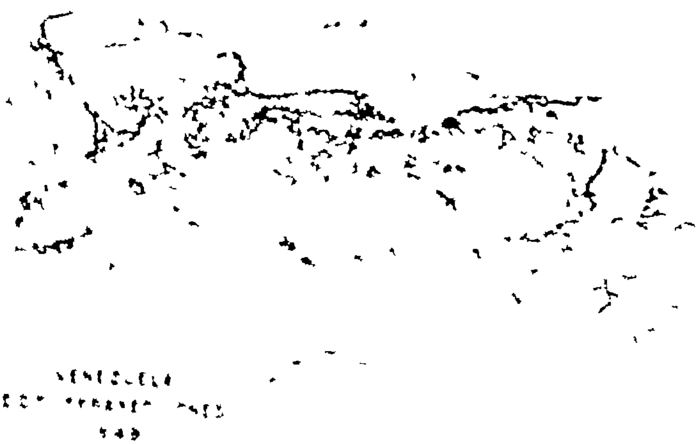

TABLE XI.

EFFECT OF DDT ON SPLEEN INDEXES ACCORDING TO THE RATIOS OF ENDEMICITY AND EPIDEMICITY IN SOME PUEBLOS OF THE STATE OF CARABOBO.

Pueblo.	First sprayed.	Ratios of		1941–1945		1946.	1947.	1948.	1949.
		Endemicity	Epidemicity	Largest.	Smallest.				
Guacara	—	1	7	33.7	1.1	47.6	15.3	4.5	3.1
Güigüe	6/1949	1	7	37.2	2.6	35.6	14.2	3.2	2.4
Patanemo	4/1947	3	6	85.9	16.0	79.3	76.2	10.1	2.0
Morón	12/1945	15	1	98.6	2.7	65.1	46.9	31.2	15.5
Urama	12/1945	14	1	92.6	70.6	78.6	41.1	30.9	13.4

Unsprayed control.

in the evolution of the spleen indexes after DDT spraying in relation to the ratios of endemicity and epidemicity is also observed in the size of the average spleen (Table XII). The average spleen size in Patanemo was practically as high as that of Morón and Urama when the highest spleen index was taken, but 2 years after DDT spraying it was as low as those of Güigüe and Guacara, the two epidemic towns. In Morón and Urama hyperendemic areas, 3 years after the spraying the average spleen sizes were 10 times higher than those of the other towns. The average enlarged spleen, calculated only on positive cases, does not reflect the influence of DDT as well as the other two indexes, probably because of different individual reaction to spleen reduction, as it is possible that some persons remain with their spleen unaltered in size for longer time than others.

TABLE XII.

EFFECT OF DDT ON THE AVERAGE SPLEEN AND THE AVERAGE ENLARGED SPLEEN ACCORDING TO THE RATIOS OF ENDEMICITY AND EPIDEMICITY IN SOME PUEBLOS OF THE STATE OF CARABOBO.

Pueblo.	Largest in 1941–1945.		1946		1947		1948.		1949	
	Average spleen.	Average enlarged spleen.	A.S.	A.E.S.	A.S.	A.E.S.	A.S.	A.E.S.	A.S.	A.E.S.
Guacara†	0.30	0.9	0.20	0	0.10	0.6	0.03	0.6	0.02	0.8
Güigüe	0.30	0.9	0.70	0.6	0.10	0.6	[illegible]	0.5	0.03	0.7
Patanemo	1.30	1.4	0.80	1.0	0.43	0.8	0.40	1.2	0.03	1.0
Morón	1.60	1.6	0.60	1.2	0.40	0.8	0.40	1	0.20	1.5
Urama	1.40	1.5	1.00	1.2	0.6	1.3	0.50	1.8	0.20	1.1

For ratios of endemicity and epidemicity of these pueblos see Table XI.

† Unsprayed control.

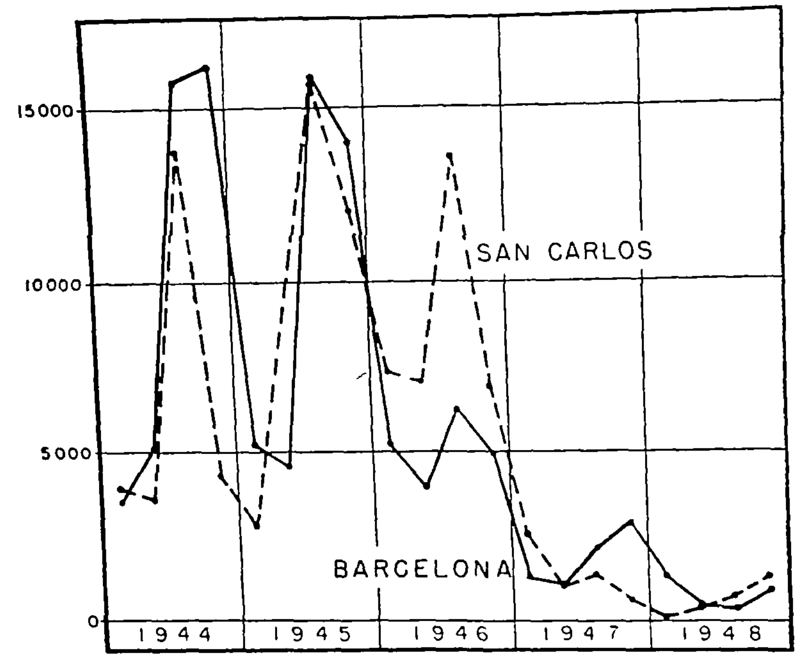

FIG 14—Malaria reduction after DDT house spraying (since November, 1946 in Barcelona, Anzoátegui and since December, 1946 in San Carlos, Cojedes) Observe that the dry season morbidity rates in the early months of 1947 were much lower than the rates of former dry seasons indicating that in the normal dry seasons there is reduction and not interruption of transmission

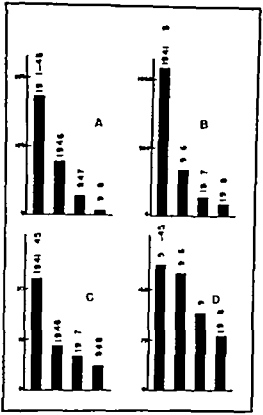

Fig. 1.—Effect of DDT house-spraying on some malaria indexes and rates. A. Malaria death rate. B. Positive slides per 100,000 house visits, C = Parasite infection index, and D = Treatments per 1,000 inhabitants. (See figures in Table XVIII.)

made yearly and slides were taken only from those found in bed. Later when the number of cases decreased, slides were taken from every person found with fever or reporting fever in the last 7 days. With the slides so obtained an index has been established, positive slides per 1,000 or 100,000 house visits, which permits comparison for different years. Another example of what is happening to malaria in Venezuela after DDT spraying based on this index, is presented in Table XIII. Similar data have been obtained from other areas. In the period 1941 to 1945 *in three States (Aragua, Carabobo and Yaracuy)* with about 500,000 inhabitants, the average of this index was 1 083 and it came down after spraying to 82 in 1948. Such indexes refer to both sprayed and unsprayed zones, the figures being lower if only the sprayed areas are considered. Fever patients from these three States gave an average index of infection with malaria parasites of 33 per cent. in 1941 to 1945. This index came down to seven in 1948, a figure which, according to MISSIROLI (1947) indicates that malaria transmission has been interrupted.

An interesting observation has been made in connection with persons with malaria parasites *in sprayed localities*. Generally in each sprayed place some houses are left untreated. In Table XIV the index of positive slides per 1,000 house visits indicates that malaria infection is very low in sprayed houses, and high in the unsprayed ones. It must be noticed that the unsprayed houses may lie between two sprayed ones. This finding has a great epidemiological significance as it is a new proof that malaria is a house infection. It also may imply that some of the cases found in sprayed houses may have become infected in the unsprayed ones. And from the standpoint of a malaria campaign with DDT it means that all the houses have to be sprayed if eradication is the objective. It should be observed *that the difference in Maturín, Monagas,* is not very high because in this town a large drainage scheme has been carried out, reducing considerably the malaria prevalence.

TABLE XIV

EFFECT OF DDT ON MALARIA PREVALENCE IN 1948 IN SPRAYED AND UNSPRAYED HOUSES OF THE SAME LOCALITY

Towns.	Malaria morbidity rate the year before spraying	Date of 1st DDT spraying.	Number of house visits	Slides with malaria parasites per 1,000 visits to	
				Sprayed houses.	Unsprayed houses.
Barcelona, Anzoátegui	9 27	Nov. 1946	22,183	1 0	24 2
C. Bolívar, Bolívar	0.24	July 1947	104 701	1 2	28·1
Guanare, Portuguesa	6,853	Apr. 1947	77 743	6 8	143 8
Maturín, Monagas	1 923	Jan., 1947	43,790	0 3	1 1

In Table XV data on the evolution of the parasite formula for different years and town are given In Acarigua, Portuguesa, *P falciparum* started to have a prevalence lower than that of *P vivax* in 1945 This town has not been sprayed, but a large drainage scheme was carried out, and the breeding places of *A darlingi* were eliminated, this being the first time that this vector has been eradicated by drainage (BERTI, 1949) In this year there was a marked decrease in the house density index of *A darlingi* accompanied by a manifest drop in the malaria morbidity rates (Table XVI) In Barcelona, Anzoátegui and in Maturín, Monagas, the increase of *P vivax* over *P falciparum* occurred before DDT spraying In these two towns there was a sudden drop of *A darlingi* in 1946 (Table XVII), the origin of which is still unknown, although influenced by drainage in the latter town The decrease of the population of *A darlingi* means a reduction of transmission which explains the change in the parasite formula In Guanare, Portuguesa, and in San Carlos, Cojedes, *P falciparum* is still more common after spraying than *P vivax* This may be due to houses left without spraying (Table XIV) or to outside influences The parasite formula of other places follows these patterns, and the change over from *P falciparum* to *P vivax* has the same meaning as in other countries, malaria reduction

That malaria has decreased after DDT spraying is also shown by the epidemic index Every week the health units and dispensaries, maintained by Federal and State funds, report the number of cases of notifiable diseases, among them those of malaria With these figures the epidemic index for the year has been calculated with the average for 1941 to 1945 as a base Table XIII shows that this index for the three States referred to above has dropped from 100 to 16 This fall has been proportionately larger in the third year compared to the second, than in the second compared to the first year Therefore,

TABLE XV

PARASITE FORMULA IN DIFFERENT TOWNS OF VENEZUELA

(FOR DISCUSSION SEE TEXT)

Year	Acarigua, Portuguesa			Barcelona, Anzoátegui			Guanare, Portuguesa			Maturín, Monagas			San Carlos, Cojedes		
	F	*V*	*M*	*F*	*V*	*M*	*F*	*V*	*M*	*F*	*V*	*M*	*F*	*V*	*M*
1941	55	42	3	57	35	7	52	44	5	50	46	4	56	38	6
1942	49	48	3	55	40	5	53	44	2	50	43	7	62	34	4
1943	50	48	2	43	52	5	61	33	6	51	41	8	52	35	13
1944	66	29	5	58	41	2	26	67	7	52	39	10	60	35	4
1945	46	50	4	41	57	2	45	46	9	62	35	3	57	42	1
1946	33	59	8	42	57	1	55	42	2	38	60	2	59	40	1
1947	33	63	4	40	58	2	50	48	2	44	56	0	42	57	1
1948	20	80	0	33	63	4	48	46	6	28	72	0	49	47	4

F = *P falciparum* *V* = *P vivax* *M* = *P malariae*

TABLE XVI.

EFFECT OF DDT ON MALARIA MORBIDITY RATES AND *A. darlingi* DENSITY INDEX.

Year	Acarigua, Portuguesa, not sprayed, drained.		Barcelona, Anzoátegui, 1st sprayed Nov 1946.		Guanare, Portuguesa, 1st sprayed Apr 1947		Maturín, Monagas, 1st sprayed Jan., 1947.		San Carlos, Cojedes, 1st sprayed Dec., 1946.	
	A.	B.	A.	B.	A.	B.	A.	B.	A.	B
1941	8 061	101	12,112	50	9 760	84	4,100	20	9 707	170
1942	6 179	29	11 437	161	10,470	373	5 035	33	11 640	1,204
1943	7,286	133	9 111	209	7 796	144	3,430	133	8,160	801
1944	4,217	171	10,116	373	11 765	175	4,406	64	6 392	1,700
1945	1,607	12	9 937	374	5 095	70	3,033	30	9,293	307
1946	964	2	5,164	2	3,855	54	1 073	0 4	5 748	114
1947	433	0	1 023	1	5 035	10	450	0 1	1,239	42
1948	220	0 0	757	0	1,719	0	85	0	620	0·7

A = Malaria morbidity rate (microscopical diagnosis) per 100 000;
B = *A. darlingi* adult density index (houses).

private doctors reporting through the health units, and dispensaries, are receiving less malaria cases after the use of DDT

In 1937 the División de Malariología organized a service of drug distribution through post and telegraph offices and schools. Quinine was used until 1944 when mepacrine was found cheaper and just as good. Any persons going to such places suffering from fever or having fever patients in his house, may get sufficient drugs for a course of treatment, in an envelope with instructions. More than 2,500 such posts have been established in the country In the three states of our example the number of treatments given per 1,000 inhabitants per year in 1941 to 1945 had an average of 187 The number fell to 81 in 1948 There is no doubt that many non malarious fevers have been and continue to be treated with these drugs, but the reduction in the number of treatments given is one of the most significant indexes of malaria reduction, because it is a demonstration of the well being of the people The average number given in 1941 to 1945 was 578,838. This number went down to 575,839 in 1946, to 457,883 in 1947 and to 381,368 in 1948. This has been the first item to be reduced in our budget

Finally there is a spectacular fall in the malaria death-rates. In the three States of the example death registration is better than elsewhere (GABALDON and DE PÉREZ 1946), and 62 5 per cent of deaths are certified by doctors. The malaria death-rate per 100,000 has declined from an average median of 173 in 1941 to 1945 to five. This great reduction is also seen in other areas. For Venezuela as a whole the malaria death-rate fell from an average of 112 2 in 1941 to 1945 to 14 8 in 1948 (Preliminary figures.)

shows the great correlation between the density indexes of *A. darlingi* and the malaria morbidity rates.

This reduction of *A. darlingi* has brought the practical disappearance of the species, because it is accompanied also by a decrease in the larval population (Table XVII). In this table it may be noticed that the larval density indexes of *A. darlingi* are smaller than those of *A. albimanus* while the house adult density indexes are larger for *A. darlingi*. This phenomenon is typical of these species throughout Venezuela, and it may indicate that the absolute population of *A. darlingi* is ordinarily smaller than that of *A. albimanus*, and therefore that the former species is more liable to be eradicated as a consequence of an

A DARLINGI DISTRIBUTION

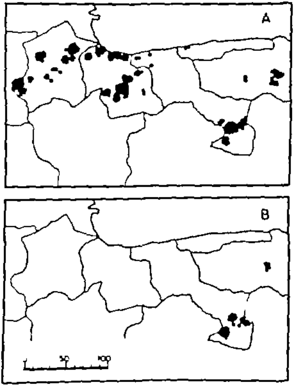

FIG. 16.—Apparent eradication of *A. darlingi* by DDT house spraying. A = Localities where *A. darlingi* had been found before spraying, and B = Localities where *A. darlingi* was found in 1948.

important reduction in its adult population. *It seems at present that actual eradication of A. darlingi has been attained from large areas as shown in Fig. 16.* But before one can have absolute confidence in these results, it is necessary to wait for the next peak of the non-annual cycle of that species, which will occur in 1950 to 1952. A similar eradication of *A. darlingi* in British Guiana has been reported by GIGLIOLI (1948).

In Table XVII it is interesting to observe the effect of DDT on *A albimanus* There was a great drop in the adult density-index after DDT in 1947 and 1948 The larval population showed a marked decrease only in 1948, but it may be a natural fall in the period 1945 to 1948, as a similar one was observed in the period 1941 to 1944 It seems, therefore, that DDT is not an agent of reduction for *A albimanus*, a fact which was expected as this vector is very zoophilic, and consequently adults are not affected in large numbers by DDT house spraying Now, the *A albimanus* house adult density indexes for 1947

TABLE XVII

EFFECT OF DDT SPRAYING ON *A albimanus* AND *A darlingi* IN BARCELONA, AN OATEGUI (SPRAYED NOVEMBER, 1946)

Year	Larval density index (all breeding places)		Adult density index (houses)	
	A albimanus	*A darlingi*	*A albimanus*	*A darlingi*
1941	422	22	83	59
1942	165	24	29	181
1943	188	31	34	208
1944	75	18	53	272
1945	447	18	108	274
1946	163	9	157	2
1947	144	1	10	1
1948	10	0	6	0
*	5,359/5,726	5 359/408	10,482/4,306	10,482/7,049

* The first figure indicates the number of standard visits to breeding places or capture stations and the second the number of larvae or adults caught in the period 1941–1948

and 1948 in Barcelona, Anzoátegui (ten and six respectively in Table XVII) with a malaria morbidity rate of 1,823 and 757 (Table XXI), are similar to those of Maracay, Aragua, in 1941 and 1942 of 22 and 4 with higher malaria morbidity rates of 3,269 and 3,196 In the latter town *A albimanus* is the only vector, and as such low densities of this mosquito here produced higher morbidity rates, it means that in Barcelona more malaria would have been expected in 1947 and 1948 if it were not for DDT Therefore the effect of DDT on *A albimanus* should be considered only as result of interception This is important, because in the literature statements are often found indicating that DDT is only an anti-malaria agent because it is a vector-reducing factor, which is not actually the case with all species It follows that the action of DDT should be judged from the point of view of malaria reduction and not from its effects on the vector population alone

The larval population of other species has not been affected by DDT Repeated examples exist in our studies indicating that the house adult-density

indexes are very low after DDT spraying while the larval density indexes are still high. In Table XVIII larval density indexes are given of some species, several of which are confirmed vectors in other neotropical countries. They do not all of them come into houses as all are zoophilic in different degrees. In this table it may be noticed that in 1948, with exception of three species, the density indexes are higher than the lowest found in 1941 to 1945 which indicates that DDT does not influence these species at all. We cannot say at the present time whether the larval reduction observed in *A. oswaldoi*, *A. pseudopunctipennis*

TABLE XVIII.

LARVAL DENSITY INDEXES OF SOME ANOPHELINE SPECIES BEFORE AND AFTER DDT SPRAYING.

Species.	Town.	Larval-density indexes.			
		Lowest 1941 1945	1946.	1947	1948.
A. albitarsis	S. Carlos, Coj.[1]	27 7	40 8	10 6	41 8
A. argyritarsis	Guanare, Port.	50 8	170 0	149 4	83 6
A. neomaculipalpus	S. Carlos, Coj.	3 1	2 3	17 6	18 1
A. oswaldoi	Maturín, Mon.	126 8	125 6	94 6	91 2
A. pseudopunctipennis	Barcelona, Anz.	178 1	130 9	951 2	96 2
A. punctimacula	S. Carlos, Coj.	0 4	9 8	4 1	9 3
A. rangeli		31 1	110	85 6	49 9
A. strodei		3 7	84 8	43 8	39 8
A. triannulatus	Maturín, Mon.	30 0	40 9	23 2	17 9

[1] DDT since Dec., 1946. [2] DDT since Apr. 1947. [3] DDT since Jan., 1947. [4] DDT since Nov. 1946.

and *A. triannulatus* is the effect of DDT house spraying. It is possible, however that this may be the case at least with *A. pseudopunctipennis* the most domestic of the three species, as such reduction has been reported from other countries.

COLLATERAL EFFECTS.

It is known that malaria reduces birth rate. The following example from the State of Carabobo (GABALDON and DE PÉREZ, 1946) is a confirmation of that fact for Venezuela

Year	Malaria death rate.	General death rate.	Birth rate.	Vital index.
1940	100 3	21 3	38 6	172
1941	423 8	25 9	37 4	144
1942	431 2	4 6	34 8	141
1943	190 8	20 7	33 9	164
1944	140 4	21 6	37	172
1945	109 4	18 7	37 9	202

There is a lag of 1 year between the rise of the malaria death-rates and the fall in the birth-rate, the result of the late registration common in recording births in Venezuela The general death-rate was also augmented, and consequently the vital index decreased, to increase later when the malaria epidemic subsided The marked decline in malaria after DDT spraying should therefore produce an increase in the birth-rate This is just what has been observed in Venezuela as shown by the figures for the three States presented in Table XIII The birth-rate has risen to 41 9 in 1948 from an average of 36 2 in 1941 to 1945

As mentioned in Section II, when the malaria death-rate comes down the general death-rate drops also, not only as the direct effect of malaria reduction, but also from a decrease in the death-rates due to other diseases This explains why the decrease of the general death-rate for the three States (Table XIII) is much larger than that expected on account of malaria reduction alone, 6 1 instead of 1 7 per 1,000 It should be observed, however, that the decline in the general death-rate reached its lowest point in 1947 while malaria continued to decrease in 1948 This will be explained later

The infant mortality rate dropped also after the introduction of DDT, as shown by the example of the three States (Table XIII) This decline, however, reached its lowest point in 1947, as did the general death-rate, in spite of the fact that malaria continued to subside in 1948 The increase in the infant mortality rate in 1948 is more marked when the absolute figures are studied, as the increment in the number of births referred to above produces a reduction in the infant mortality rates These absolute figures are as follows, for the three States of Aragua, Carabobo and Yaracuy

Years	Number	Years	Number
1941–1945 (average)	2 291	1947	2 041
1946	2,098	1948	2 207

Therefore, the increase in infant mortality in 1948 is very significant and is related to the change in trend that the general mortality showed in that year

After DDT spraying it was observed in Italy (MISSIROLI, 1948) that malaria mortality was not the only one reduced, but also that the summer increase in the number of deaths produced by diarrhoea and enteritis disappeared This has been reported also from other countries, the explanation being that the reduction in the fly population causes an interruption of the transmission of disease by them, and in Table XIII it is seen that a very pronounced reduction in the death-rate due to diarrhoea and enteritis was obtained However, this decline only lasted until 1947, and was followed by an increase in 1948 This appears to be connected with the apparent resistance developed by flies to DDT If

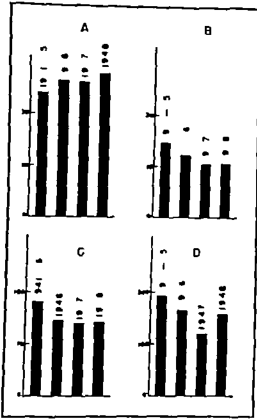

Fig. 17.—Possible collateral effects of DDT house-spraying. A = birth rate; B = general death rate; C = infant mortality rate; D = diarrhoea and enteritis death rate. (See figures in Table XIX.)

this is an adequate explanation, the small increase in the general death-rate and the marked one in infant mortality in 1948, are possibly the consequence of the present lack of effect of DDT on flies.

These collateral effects have undoubtedly been partially due to the recent economic well being of Venezuela, as well as to DDT but the part played by each cannot be unravelled. Certainly the decrease in deaths due to diarrhoea and enteritis showed an original potent effect of DDT but the effect of economic improvement throughout Latin America should not be discounted. Birth- and death-rates of some Latin American countries are shown in Table XIX. In Chile, malaria was prevalent in a small district and never was important. In Colombia, Costa Rica, El Salvador and Mexico malaria is as important as it used to be in Venezuela, and no nation-wide campaign against it has been carried out. In all of these countries the general death-rate has decreased significantly in the last years. In spite of the fact that Venezuela had the lowest death rate in 1947 the rate of decline of Chile and Mexico was higher. The only difference between Venezuela and the other countries seems to be the constant increment, without fluctuations, of the birth-rate. With these facts at hand it may be concluded that great care should be taken in judging the possible collateral effects of DDT house spraying in a country.

Other Effects

DDT toxicity to man, to domestic animals, and to other insects, deserves some attention. There have been 12 men working all the year around since the beginning of 1946 who still are with the squads and have not presented any

TABLE XIX

BIRTH AND DEATH RATES OF SOME LATIN AMERICAN COUNTRIES TO SHOW IMPROVEMENTS IN PUBLIC HEALTH

(STATISTICAL OFFICE OF THE UNITED NATIONS)

Country	1939			1944			1945		
	Birth rate	Death rate	Vital index	Birth rate	Death rate	Vital index	Birth rate	Death rate.	Vital index.
Chile	35 2	24 6	143	33 2	19 5	170	33 3	20 0	166
Colombia	31 6	17 6	180	32 4	16 4	198	31 8	15 8	201
Costa Rica	42 3	18 3	231	41 3	15 6	265	43 6	14 4	303
El Salvador	41 1	18 1	227	37 5	17 5	214	37 7	16 0	236
Mexico	44 6	23 0	194	44 2	20 6	215	44 9	19 5	230
Venezuela	35 9	18 7	192	35 9	17 2	209	36 8	15 3	240

Country	1946			1947		
	Birth rate	Death rate	Vital index	Birth rate	Death rate	Vital index
Chile	32 4	17 2	188	33 8	16 7	202
Colombia	33 0	15 6	212			
Costa Rica	41 7	12 9	323	45 3	14 2	319
El Salvador	36 1	15 5	233	41 2	15 0	275
Mexico	42 5	18 7	227	45 1	16 3	277
Venezuela	38 4	15 0	256	39 5	13 9	284

signs of intoxication They were supposed to work with a mask and impermeable gloves, but in practice most of them did not use this equipment because it was cumbersome The men change their uniforms three times a week and are advised to wash the hands well before taking food and to have a bath at the end of the day While working with kerosene solutions some men have complained of conjunctivitis, dermatitis and/or pharyngitis, and some had to be relieved from work on account of these effects which were more due to kerosene than to DDT Vasomotor rhinitis has been observed in other workers and in people from houses sprayed with solutions This rhinitis, however, disappeared within 1 month after the spraying Toxicity to man, other than these possibly allergic reactions, is unknown in our experience

Cats and chickens have died of what appeared to be DDT intoxication, and mice and geckos have been found dead after spraying Cats and mice may die from DDT they get on their skins, and chickens from eating poisoned cockroaches Geckos are susceptible to DDT as are other cold-blooded vertebrates Chickens washed in 5 per cent DDT suspension to kill lice are not

poisoned, although the DDT is allowed to remain on the feathers. Some householders say that cats die after eating poisoned mice.

Flies were killed readily by DDT at the beginning of the campaign. Cattle farmers, especially were happy and co-operative after DDT spraying of stables and cowsheds. The number of flies, however increased later and many people believe that the present DDT is not as good as it used to be. Flies disappear from the houses for some days after spraying with kerosene solutions, but after about 15 days they are as abundant as before. Experiments carried out in our research laboratory by Dr JOHN MAIER indicate that native fly strains may develop DDT resistance as early as the second generation.

Aëdes aegypti is eradicated from sprayed towns, and reinfestation occurs 1 year after the spraying With the large-scale spraying carried out in Venezuela, this species has disappeared from large areas and the anti-aegypti eradication programme which the División de Malariología is also carrying out, has been greatly helped. *Culex quinquefasciatus* is found inside houses sooner than other mosquitoes after spraying This species is more resistant to DDT than other culicines, an observation also made in other countries.

An interesting phenomenon observed 1 or 2 years after the start of the DDT house spraying programme is a reported increase in the density of *Rhodnius prolixus*. Householders from different rural sections of Venezuela started to complain that these triatomids were more abundant than before the spraying, and some people say that this increase is due to the killing of geckos which eat the triatomids. Another possible explanation may be the disappearance of other insects which may eat the eggs and larvae. Laboratory experiments from the Section of Special Studies (Dr JOHN MAIER) show that these bugs are resistant to DDT in the amounts used for anopheline interception.

DISCUSSION.

The objects of malaria control by anti mosquito measures have been divided by the writer (GABALDON, 1948) into four groups eradication, reduc tion, exclusion and interception of the anopheline vectors. The control of malaria may involve the reduction of the disease or its eradication. In the past, the goal was malaria reduction obtained by exclusion or reduction of the vectors. Eradication of the vectors is naturally followed by eradication of malaria, but it has been attained only in very limited regions. Interception of the vector has greatly increased the possibilities of malaria eradication and the goal of a campaign against this disease at the present time is its total elimination even in tropical countries.

In the early days it was thought that DDT was only an agent of inter ception, a compound like pyrethrum, capable of killing anophelines during the extrinsic incubation period of the malaria parasites, producing the inter ruption of transmission. But present experience shows that it may also fulfil the other three objectives.

DDT seems, at least in some cases, to be a repellent, a chemical means of exclusion The repellent effect of DDT is hard to explain, but at the same time it cannot be proved from field observations that under some conditions it does not exist On the contrary, our experience has generally shown that the anopheline population disappears from sprayed houses while still prevailing in neighbouring unsprayed ones The very fact that at least one spraying squad, when arriving late in the day at malarious places, developed the method of dusting the floor of the sleeping rooms with wettable powder, instead of spraying the walls according to the regulations, probably is a good example of the repellent action of DDT It should be noticed that the repellent effect is not produced by kerosene, but by DDT itself, as the inert materials of the wettable powders do not seem to be responsible for it But this repellent action does not last as long as the intoxicating power of DDT, because 2 or 3 months after the spraying, mosquitoes frequently start to appear in larger quantities in the houses, and many of them are found poisoned or dead If DDT is a repellent, its anti-malaria effectivity may be enlarged to include those vectors which come into the houses to bite but do not remain long in them This repellent action, however, should not be confused with the effect apparently produced by DDT in some species, which may inhibit the mosquitoes from alighting or remaining on sprayed surfaces, forcing them to leave the house This action, by exposing the vector to unusual risks in its life cycle after feeding, may bring a high mortality, eliminating the infected ones, an effect which might be considered as another example of interception

The decrease of the anopheline population after DDT spraying, not from houses alone but also from breeding places, shows that it is an agent of mosquito reduction, at least for some species Nevertheless, there are anophelines whose larval population is not diminished by DDT house spraying, although a decrease of the adults in human dwellings is observed This decrease should not be considered as mosquito reduction, and the control of malaria which may be attained in these places by residual DDT is the result of interception or exclusion of the species This difference is important to bear in mind, as in those regions where true reduction of the vector is obtained, the use of DDT may be reduced, lowering for the time being the cost of protection

Eradication of *A darlingi* seems to have been obtained in some regions of British Guiana and Venezuela This is a highly domestic and anthropophilic species, which can be greatly affected in its life cycle by DDT The great diminution in the number of eggs laid in the breeding places is possibly responsible for the inability of this species to maintain itself in the sprayed zone It must be remembered that *A darlingi* does not reach high densities in its breeding places, apparently needing more water surface than other species, a factor which may help in its elimination. It is not known at the present time whether this eradication of *A darlingi* is really occurring, or whether the observed decrease is due only to a low point in its cycles of range fluctuation and

population density Its relatively easy disappearance after DDT spraying may also be due to the fact that Venezuela and British Guiana are not in its genetic areas of dispersion. If true permanent elimination of this anopheline is to be obtained the campaign with DDT should include the spraying of all houses.

The interception of the anopheline vector although introduced 40 years ago by CHAGAS (1908) who proved that malaria was reduced with sulphur fumigation of houses, is a relatively new concept in anti malaria work. Although the term was proposed by the writer (GABALDON 1948), it was clearly defined by DE MEILLON (1936) when he stated that the whole idea underlying malaria control work by anti-adult measures is the killing of the infected vector and not of all the mosquitoes of the vectorial species. In addition to the eradication and reduction of the arthropod host by DDT and of its possible exclusion by the same agent the writer believes that more thought should be devoted to this problem of interception of malaria transmission. Probably *A albimanus*, *A albitarsis* and *A aquasalis* and other species whose larval populations do not appear to be reduced by DDT are made unable to transmit malaria only by killing the mosquitoes which received an infective meal. The time anophelines require to become poisoned by DDT inside the houses may be much shorter than the time they usually remain in the human dwellings.

The interception of the anopheline vector may be a less effective measure than its reduction. For this reason it is not advisable in this type of work to start with the classical two villages, one sprayed with DDT for the experiment and the other unsprayed for control. The importation of gametocyte carriers into the treated village may nullify the results as it is always possible that some mosquitoes may survive the time required for the extrinsic incubation period. It is therefore convenient to begin with an infected zone near the sea, or isolated from other malaria areas by hills or other topographical accidents, of a size from 500 to 1,000 square km. The comparison of the parasite rates in younger children, especially the new born, before and after spraying, or with those of a neighbouring area of similar malaria prevalence may be the only measure required to see if malaria has been reduced or not.

It should not be forgotten that the main object of DDT house spraying is to produce a decrease in malaria prevalence There are already papers in the literature coming from different parts of the world where more attention is paid to the action of DDT on mosquitoes than to its effect on malaria reduction. This tendency may bring confusion and hamper the establishment of effective malaria control work in regions which badly need it. DDT may influence in a different way different species of anophelines, and this fact must be remembered before reaching definite conclusions. Because of this fact, the effect of DDT on malaria transmission by a given species cannot be presumed from preliminary bionomic studies alone. Malaria reduction should be the only measure of DDT effectivity Therefore, it is our belief that nothing short of measuring the effect of DDT in terms of malaria prevalence will give an answer

[illegible] It is probably in its power as a [illegible] control agent in a treated locality. [illegible] DDT is useful or not [illegible] the simplest, quickest and cheapest method to [illegible] *A. [illegible]* and [illegible] in a given area. In [illegible] of Venezuela, where *A. [illegible]*, DDT house spraying [illegible] however, do not [illegible] at the present time. [illegible] should [illegible], they only mean [illegible] the world, the [illegible] DDT [illegible] the present [illegible] should not [illegible] DDT on the [illegible]

[illegible] has been [illegible] may be applied [illegible] the concepts may [illegible] which [illegible] In many instances [illegible] will [illegible] may be the only [illegible] only when the [illegible] high enough to pay attention [illegible] control [illegible] malaria control work people have already acquired [illegible] human [illegible]

Guided by these [illegible] in 1945 we undertook [illegible] the DDT on a nation-wide scale in Venezuela. The experience obtained from 1936 to 1945 in the study and control of malaria and its vectors in this country allowed the planning on a large pattern with a probability of success. The two main vectors, *A. albimanus* and *A. darlingi*, were known [illegible] their [illegible] power [illegible] endemicity and epidemicity, [illegible] The geographical distribution of malaria, and its [illegible] were also understood. The trend of the disease [illegible] known. With such a background we felt confident to go ahead in our enterprise.

In the example presented in the section on results, it was seen how radically the whole picture of malaria in Venezuela has changed after DDT house spraying. From large zones, *A. darlingi* seems to be in the process of eradication. Wherever DDT has been applied, malaria has subsided. The general health indexes of the country have improved. The prospect of total elimination of the disease from the most important region is contemplated. The outstanding obstacles to our objective have been the low density of the population and poor roads in rural areas. To overcome these difficulties has required a great deal of effort, and on their final solution will depend the success to the goal ahead of us: eradication of malaria from Venezuela. But, if these physical problems are significant obstructions on our way, there are others, lying more on the psychological side, which should not be overlooked. It is hard for malariologists in direct charge of field work to grasp that a few cases of malaria are too many. To people

used to finding hundreds of positive slides each month five to 20 new cases have the same meaning they think that malaria is decreasing and disregard *carrying out a survey to inquire why these cases exist at all.* We have repeatedly emphasized that new malaria cases, in any area protected for more than 1 year with DDT need an epidemiological investigation as complete as the one carried out by any city health department in reference to cases of diphtheria or typhoid fever On this action will depend the eradication of malaria and the end of the DDT house-spraying campaign. We sincerely think, based on our present experience, that this objective may be attained under at least neo-tropical conditions. This, however by no means conveys the idea that malaria control staff will be without work some day as the large experience acquired will permit the development of larger programmes of house disinfestation, a task long enough to keep us busy the rest of our lives.

In the campaign with DDT against malaria in the tropical zones of the world it seems that two stages are going to be followed (1) reduction of the disease, and (2) its eradication. Similar steps were taken in the control of *Aëdes aegypti.* Reduction of this mosquito, however has followed two different paths. One has been based on systematic work as was carried out in Brazil since the first years of the yellow fever campaign. The other has been indiscriminate action by unspecialized agencies as is practised in the United States of America and in many Latin American republics. The last method has shown to be expensive and incapable of safeguarding a town against yellow fever If due care is not taken at the present time, anti malaria programmes with DDT will follow similar lines. The mistake is to let the public health authorities believe that malaria can be eliminated without specialized technique, as some of them, under-estimating the inherent difficulties, are now preaching that malaria is a problem already solved. But the systematic reduction of malaria should only be a step towards its eradication, and proper studies of the methods and administrative procedures to be followed should be undertaken as soon as possible. It is true that some tropical countries will be unable to start a large-scale scheme at the very beginning, but the reduction of malaria obtained if the available resources are adequately used, will help in developing new resources with which to enlarge and increase the programme.

A nation-wide DDT house-spraying campaign in a tropical country is not a simple undertaking It is true that the spraying of houses with DDT is relatively easy work, but on a large scale it is a difficult and costly enterprise. The *elimination of malaria from* large portions of the tropical regions of the world will depend on the intensity of the campaign. There are many obstacles to the maintenance of a long term nation-wide DDT house-spraying programme. It is therefore important that, in spite of the possibility of being a short lived profession, malariologists of high standing continue to be produced. Otherwise years will go by and will see malaria unchecked or only slightly reduced, calling for a continuous expenditure which in the end may require much more money

With our present experience no goal short of malaria eradication should be aimed at But this will finally depend on the conviction of malariologists themselves that it can be done, and the enthusiasm that they devote to it, the most important public health activity in a large part of the world

SUMMARY

Venezuela is considered divided into three regions Costa-Cordillera, Llanos and Guayana, which are different in topographical, meteorological, social and economic characters Malaria, as a result of the topographical and meteorological conditions, has played an important role in creating the difference between these regions

The main vectors of malaria in Venezuela are *A albimanus* and *A darlingi*, the first species being found mostly in the Costa-Cordillera, and the last one in all the three regions with different degrees of prevalence *A albimanus* is in part zoophilic , *A darlingi* is mostly anthropophilic and a house resting mosquito As a result they react differently to DDT house spraying *A albimanus* is intercepted and its larval population is apparently not reduced *A darlingi* is reduced and may be eradicated

Past studies show that the endemicity of malaria is low, with relatively small areas of hyperendemic malaria, and that its epidemicity is generally high This is due to the fact that the two main vectors are less potent than the most important Ethiopian or Oriental ones This epidemic tendency is particularly shown in the 5-year cycles of the disease, which appear to be connected with similar cycles in range fluctuation and population density of the vectors, especially *A darlingi*, cycles which are common, though with different periodicities, to other neo-tropical species

The División de Malariología has organized an intensive DDT house-spraying programme since the end of 1945 Details of the whole organization are given By the end of 1948 the percentage of houses of the malaria zone directly protected with DDT was 37 2, and probably at least 50 per cent of the houses of this zone have been influenced But as the regions with higher malaria prevalence have been already sprayed, the decline of malaria of the whole country is remarkable The malaria death-rate fell from an average of 112 2 in the period 1941 to 1945 to 14 8 in 1948

The success so far obtained leads to the possibility of eradication of malaria from the country This possibility is discussed, and attention is called to the fact that the action of DDT residual spraying should be measured only in terms of malaria reduction and not deduced *a priori* from studies of its effects on mosquitoes It is emphasized that malaria eradication from large areas of the world will finally depend on the conviction that the malariologists themselves may have that it can be done

seems to me there is one aspect of this problem of malaria prevention which is sometimes lost to sight. In countries that are afflicted with malaria it is not merely the inhabitants who suffer but also the Government. That is to say you often find that where there is a great deal of malaria it is very difficult indeed to get the authorities to move and give a free hand to the officers who could do the sort of work that Dr GABALDON has told us of I should like to ask him how he and his department have managed to impress their Government with the importance of the problem before them, so that they have secured facilities for carrying out the wonderful work I think they have done? When I listened to the speaker referring to spending 84 cents per head on this I was lost in admiration of a Government sufficiently enlightened to realize that it was worth while spending money to eradicate the disease of malaria, which not merely causes an immense loss of life but also destroys the prosperity of the people. There is a great secret in this, and I would very much like to know how we can impress administrations and governments with the importance of the malaria problem.

His Excellency Don Manuel Arocha, The Venezuelan Ambassador I think it is going to be very difficult for my friend to answer that question, and the answer is, I am afraid, because we have had the privilege of having a man like Dr GABALDON with not only his knowledge but his will power his enthusiasm and, I would say his patriotism.

Sir Philip Manson-Bahr I would like to reiterate what others have said I am full of admiration of the remarkable power of Dr GABALDON over his superiors in Government Departments, and I was struck by the very judicious way in which he put his story tonight. He has not made any extravagant claims. He possesses, if I may say so the attitude of a true naturalist because he does not instantly jump to conclusions. He has also taken due cognizance of the prevalence of those cyclical evolutions of mosquitoes of such species as *A. darlingi* and *A aquasalis* whose numbers may be reduced by natural causes and not by man-made measures of extermination. He has also considered the influence of malaria on the prevalence of other diseases. It is a self-evident fact, but not one generally appreciated, that, if you diminish the incidence of malaria you also diminish the incidence of incidental diseases, such as that of influenza, in causing a rise of malaria mortality This is the attitude of a true clinician, an attitude not always appreciated by epidemiologists. I would like to know why *A. darlingi* is especially susceptible to house spraying with DDT GIGLIOLI has made this point in his work on the eradication of malaria from British Guiana but this species did not have much attention paid to it until the last 3 or 4 years, and now has become an important malarial vector in the southern hemisphere. I would also like to know by what means

these DDT-resistant flies are produced Is the acquirement of this natural resistance by the house fly next to be imitated by certain species of anophelines? I congratulate Dr GABALDON, not only on his very vivid elucidation of this great problem in Venezuela, but also on the manner in which he has expressed it

Dr P C C Garnham I was going to ask Dr GABALDON to tell us a little about the intensity of malaria in Venezuela, about the presence or absence of zones of actual hyperendemicity By the conclusion of his paper, it became apparent that such a question today would be quite irrelevant, so successful has been control Still, before the campaign started, was malaria ever hyper-endemic in the sense that the term is used, say, in tropical Africa, where children up to the age of 9 months show practically a 100 per cent parasite rate?

We have been warned so much in the past about the dangers of DDT upsetting the "balance of nature" that it was interesting to hear of it happening—although on a small scale, *viz*, the abnormal multiplication of the triatomid bugs I should like to ask if this has resulted in any increase in the incidence of Chagas' disease

The President If nobody else wishes to speak now I feel you must all agree with me that we have had today a really delightful evening listening to this account of Dr GABALDON'S Sir PHILIP MANSON-BAHR mentioned the extremely modest claims made by Dr GABALDON, and I think if one considers the results which he has described to us so very clearly that the modesty is perhaps not quite justified I think the results obtained have been quite extraordinary considering the comparatively short period which has been spent on this nation-wide campaign Several questions have been asked Dr GABALDON, and perhaps he would like to answer

Dr Gabaldon (in reply) I thank you very much for the interest taken in my paper In reference to Dr BENTLEY'S question regarding the adequate budget of our Malaria Division, I must say that since 1936 there has been in Venezuela very active work in Public Health As a result of this fact, and as malaria was the main problem, nearly 25 per cent of the total budget of the Ministry of Health and Social Welfare was devoted to malaria control This proportion has decreased in later years, but as the total budget of the Ministry is today ten times as large as it used to be, the anti-malaria budget has been continuously increasing during all this period, until it has reached the substantial amounts referred to in the paper Sir PHILIP MANSON-BAHR was interested in the relationship that influenza and malaria had as a cause of

death during the epidemic. I must remind you that in several regions of Venezuela epidemic malaria has produced a higher total death rate than the worst epidemic of influenza the country has had, that of 1918–19. I particularly want to thank Sir PHILIP MANSON BAHR for his very generous remarks.

In reference to Dr GARNHAM's questions relative to the presence of malaria hyperendemicity in Venezuela, I must say that we do have high endemic malaria in restricted areas among zones of lesser endemicity. The presence of all degrees of malaria prevalence between epidemicity and high endemicity has been one of the most interesting epidemiological characteristics of the disease in Venezuela.

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COMMUNICATIONS

PLANNING THE CONTROL OF SLEEPING SICKNESS

BY

K. R S MORRIS, D SC,*

Medical Entomologist, Gold Coast

CONTENTS

The inhabitants of Lawra district in the north-west corner of the Gold Coast, still retain memories of a sleeping sickness epidemic that came, so they say, from French territory across the Black Volta river and swept across the western half of their country some 50 years ago The epidemic was especially severe along this river and left many of the villages with their populations decimated In 1924 the District Commissioner of Lawra-Tumu drew attention to the prevalence of sleeping sickness in his district and, as a result, a brief investigation of an area 50 to 60 square miles in extent around Lawra was made by Dr MACKAY (Gold Coast, 1925–26) Numbers of cases were found, with a marked concentration in villages within 3 miles of the Volta and Kamba

*It is a great pleasure to be able to record my indebtedness to Médecin Colonel LE ROUZIC and to members of his staff in the French West African Sleeping Sickness Service for their unfailing and kindly hospitality during my visits to their territory They have been especially generous in according me freedom of access to their reports, maps and records, from which much of the material for this paper has been gathered

rivers. It was considered that the area to the north would be more heavily infected but that there was little danger of a southward spread into Wa district.

When Dr SAUNDERS, who is now in charge of the Trypanosomiasis Campaign, was Medical Officer of Lawra-Tumu district in 1935 he found that large parts of the area were heavily infected with trypanosomiasis, the disease taking quite a spectacular epidemic form in some villages. For example, Kwala, on the upper Kulpawn river had lost half of its inhabitants through sleeping sickness in the 12 months preceding Dr SAUNDERS's visit, and 47 per cent. of the remaining 160 villagers were found to be infected. A Sleeping Sickness Camp was started at Lawra, with a well organized system of diagnosis and treatment that soon gained the confidence of the people, who brought in cases in increasing numbers. Between 1936 and 1938 2,850 patients came in voluntarily for treatment, so that when the present trypanosomiasis campaign was instituted in the Gold Coast in 1937 the position in the Lawra and Tumu districts was reasonably well known. Sample surveys of some of the heavily infected villages were made in 1938, and in 1939 survey and treatment teams followed a broad strip of country along the Lawra-Tumu and Lawra-Wa boundaries and covered the north-west quarter of Wa district.

The distribution of infections found in these surveys was compared with that of new cases coming to the treatment centres at Lawra and at Wa (started in 1939) and close agreement between the two sets of data was found up to a distance of about 30 miles from each centre. Dr SAUNDERS had found the same close correlation between survey and treatment centre returns from Gambaga district on the eastern side of the colony and he decided that once the confidence of the natives is well established the admissions of new cases at a hospital or camp give a reliable index of the extent of trypanosomiasis and of its fluctuations from year to year within a radius of 25 to 30 miles from the centre (SAUNDERS, 1938). This is a most valuable finding, since it enables the distribution of sleeping sickness to be plotted in an area served by a well-run treatment centre, and the progress of the endemic or epidemic to be followed year by year without the interference of repeated mass surveys and treatments. Further, there are reasons for considering that a treatment centre attended by voluntary patients has little effect on the local incidence of the disease. In the first place patients come in only when they feel sick or when their relatives notice recognizable symptoms, and this is after they have passed through the most infective stage. If they did not come in for treatment they would, with increasing sickness, stay more at home and so come less and less in contact with tsetse. Their treatment, then, does little to cut down the amount of infection circulating in the neighbourhood of their villages. If this hypothesis is correct appreciable fluctuations in attendances at such centres will be due to extrinsic factors, either natural, such as climate, or artificial, such as vector control. A marked seasonal rhythm in the numbers of admissions is a feature of all the established trypanosomiasis camps in the Northern Territories, attendances in the 6 months of the dry season being 20 to 40 per cent. higher than those in the 6 wet months. This corresponds closely with the activities of the people, who are too busy during the farming and harvesting period, May to October, to come in to hospital unless seriously ill, but who come more readily during the comparatively slack time from November to April. The correspondence is so exact that a local variation in their agricultural practice between the Lobi of Lawra, who take up their harvest in October and the Dagarti of Wa, who do not harvest until November is reflected in the dates at which increased attendances are noticeable in the two hospitals, Lawra being a month earlier than Wa in this respect. In view of this striking correlation it is permissible to take long term variations, in which the seasonal fluctuations are smoothed out, as indicating true variations in the amount of the disease in the locality under observation.

A study of treatment centre admissions has formed the basis for planning an attack, on entomological lines, on the epidemic in the Lawra and Wa districts of the Gold Coast and for observing the effects of the measures employed on the incidence of trypanosomiasis during the past 10 years.

I THE EXTENT OF THE EPIDEMIC

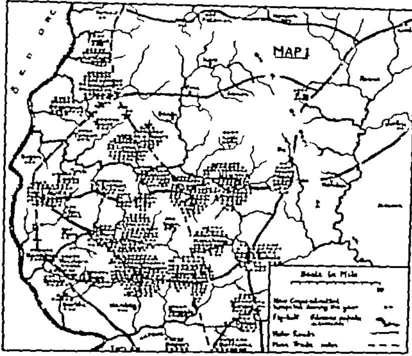
The main investigations and control operations were undertaken in Lawra district, in the extreme north-west corner of the Gold Coast. It is for the

most part a well populated, heavily cultivated district, a little over a thousand square miles in extent Investigations were extended into the neighbouring districts of Wa to the south, well farmed but less densely populated than Lawra, and Tumu to the east, a very thinly populated area, large tracts of which are completely uninhabited

This country lies in the north of the Inland Savanna Forest Zone, and shows the consequent extreme climatic variations of a 6-months dry season, from October to March, with the greater part of the 50-inch rainfall occurring between June and September Drainage is westwards into the Black Volta, which forms the international boundary with the Ivory Coast, and eastwards into the Kulpawn, flowing through Tumu and east Wa There is sharp contrast between the xerophytic vegetation of the open savanna woodland and the dense evergreen vegetation fringing the banks of these rivers and their main tributaries This evergreen vegetation constitutes permanent habitat for the almost ubiquitous *Glossina palpalis* R D and *G tachinoides* West The game tsetse, *G morsitans* (var *submorsitans* Newst) occurs in abundance in the thinly populated parts of Tumu and east Wa and is present also across the Volta in the Ivory Coast An invasion from this latter fly-belt into part of Lawra district took place in 1939, but was subsequently brought under control Because of their intimate contact with the people, whose lives are also governed largely by the presence of permanent water, the two former species are the principal vectors of the gambiense form of trypanosomiasis present All three species are important vectors of animal trypanosomiasis

Sleeping sickness is known to have existed along the Black Volta for over 50 years At the time of the arrival of the French in the Upper Ivory Coast in 1899, it was already serious in many Volta-side villages, and by 1907 was reported to be causing their abandonment, large numbers of the inhabitants having died and the remainder moving back to new sites a mile or more from the river banks But the disease was still confined to the vicinity of the main river and was being spread by canoe traffic, at that time the principal form of transport in a very unsettled country It was not until after 1920 that sleeping sickness became a menacing epidemic away from the Volta, having spread first along the courses of the larger tributaries, such as the Kamba and Bakpong rivers in the Gold Coast and the Bougariba and Bambasso rivers in the Ivory Coast In 1938, when the present investigation started and the French survey was well under way, the epidemic was found to cover more than 30,000 square miles of country, extending across the upper reaches of the three Volta rivers, with infection rates varying from 5 to over 15 per cent in different regions By that time severe depopulation had resulted on both the French and the British sides of the Black Volta and had affected the lower reaches of its main tributaries Scarcely a village remained within 2 to 3 miles of these rivers, whereas within these depopulated zones numerous ruins were to be found, dating from quite recently to over 40 years back A study of the presence of *Glossina* in relation to these ruins and to statistics of recent population declines,

gave proof of the responsibility of trypanosomiasis for the abandonment of the riverside areas and confirmed the histories obtained from the local natives and from French sources. MURAZ, 1939 (GOUZIEN 1907)

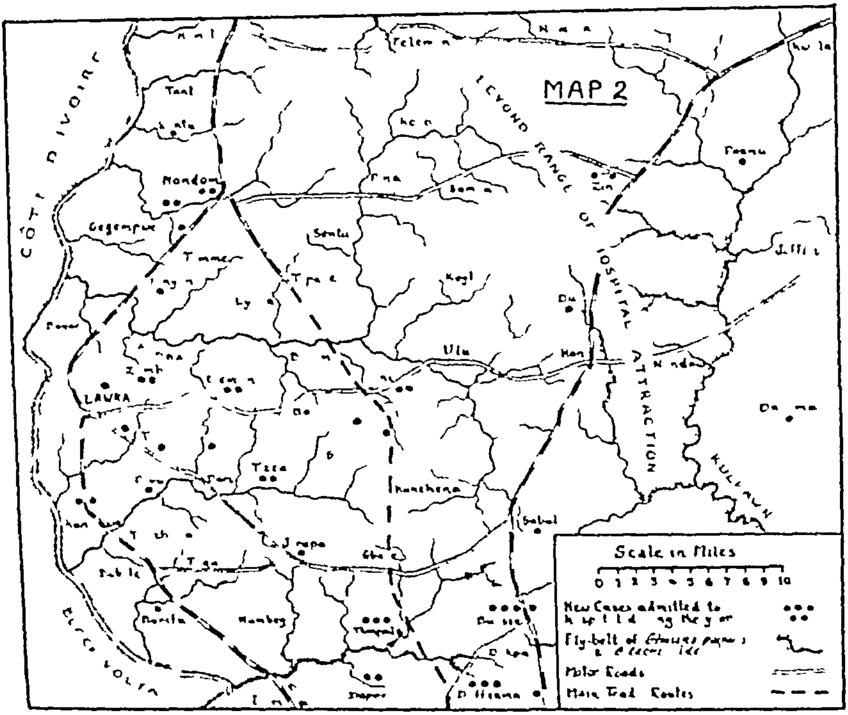


MAP 1.—Distribution of Sleeping Sickness and Dry Season habitat of *G. palpalis* and *G. tachinoides* in Lawra district in 1938 before start of control measures.

Depopulation is only in part due to direct mortality from sleeping sickness. When the number of deaths in a village becomes high the remainder of the villagers move to a new site some miles away, either on a side stream or on the high land between rivers. This movement furthers the spread of the disease and, by causing a concentration of population on hills and watersheds, brings about the serious evils resulting from overcrowding and soil erosion. When a district census was made in 1942 it was found that the watersheds around Lawra and Nandom were populated at densities above 250 per square mile which is far too high for the primitive methods of cultivation in practice. Large areas of sheet erosion were to be seen, crop yields were decreasing, and the soil of many of the hill farms was reported by the Agricultural Officer to be nearing complete exhaustion.

The distribution of sleeping sickness in 1938, compiled from the number of new cases diagnosed at Lawra Hospital during the year, is shown in Map 1. This by no means represents the full extent of the epidemic since it was known at that time that many persons were going over to the French for treatment, while a large proportion in the remoter parts of the district never came in at all. But within a radius of roughly 30 miles from Lawra the map gives the most reliable picture we have of the state of the disease at that time. It tallies

well with subsequent survey findings and it serves as a basis for comparison with later years, and for thus evaluating the effects of control measures The extent of permanent fly-belt of *G palpalis* and *G tachinoides* is also shown



Map 2—Distribution of Sleeping Sickness and Dry Season habitat of *G palpalis* and *G tachinoides* in Lawra district in 1947 after eradication of the Tsetse on the Volta Tributaries

Fly-belt is practically uninterrupted along the Volta and the lower reaches of its tributaries, with *G tachinoides* predominant and usually in very large numbers Higher up the side-streams, where the fringing evergreen vegetation thins out, the distribution of tsetse becomes discontinuous, and although the numbers are smaller *G palpalis* is proportionately more numerous than on the Volta On the upper reaches of the rivers, and on many of the smaller side-streams, fly-belt is represented by dense, evergreen groves isolated by long stretches of open river These groves are occupied by small but permanent communities of *Glossina*, with *G palpalis* often predominant or alone The map shows the principal trade routes, along which there is constant traffic, mostly on foot of traders from French territory in the north and labourers going to and from Ashanti and the coast Besides this there is constant movement of the local natives between villages on visits to markets, funerals ceremonies, etc Since the local Lobi and Dagarti tribes are spread across both

applied with success on its tributaries, treating each one down to its confluence with the Volta. A final objection arose from the fact that the Volta river formed the international boundary. Although the French were engaged in a wide anti trypanosomiasis campaign, this did not include extensive clearings on the Volta and co-operation in 1940 was unlikely. To engage in clearing on one side only of this river would have been almost futile.

There was a further argument for pursuing a policy of tsetse eradication throughout a whole district. Not only would this ensure the eventual disappearance of the disease except for introduced cases infected elsewhere but it would serve to correct the mal-distribution of population that had resulted from the abandonment of the river valleys. These river valleys offer the best farming land, good grazing all through the year and a readily available water supply even at the height of the dry season. If they could be made safe for the return of the people and their stock it would take the pressure off the over crowded uplands and, because of the greater agricultural potentialities of the land and the freedom from cattle trypanosomiasis, such a move would enable fuller and more complete agricultural development to be undertaken. The resulting improvements in nutrition and the standard of living would be reflected in the general health of the people and the eventual gain would be far more than the control of a single disease.

III Control Operations.

The method of eradicating *G. palpalis* and *G. tachinoides* by selective clearing has been described in a previous paper (Morris, 1946). Briefly the principle of eradication is based on the concept that the tsetse community on each river system forms a natural biological unit. The communities extend widely along the water-courses during the rains but contract to well defined and often very restricted foci during the adverse climatic conditions of the dry season. These foci or permanent fly-belts are confined to certain definite vegetation associations containing a limited number of species of trees and shrubs whose presence is essential for the survival of the tsetse during the hot and arid period from December to March. The removal of only these essential species of trees throughout a whole river system is sufficient to ensure the disappearance of *G. palpalis* and *G. tachinoides* since their dry season foci are now untenable for at least 4 months of the year. Clearing is thus standardized to a work of scientific precision, with a definite formula to be followed, instead of being a matter of judgment always liable to the personal equation, or of arbitrary lengths and breadths, difficult to fix and very difficult to apply over wide varieties of terrain. A number of practical advantages are gained in this way. The destruction of vegetation is reduced to a minimum, which of itself is a benefit in this semi arid country and materially lightens the labour and cost both of the initial clearing and of subsequent maintenance. There is the possi

bility of permanently eradicating the fly-belt since one is dealing with certain specific trees only. Finally, the technique is easily taught to intelligent Africans and is subject to exact and rigorous checking, points of great importance when, as in the present instance, large scale operations had to be undertaken with no additional European supervision.

The first essential for this type of eradicative clearing is the preparation of accurate maps showing particularly the rivers and streams and the distribution of permanent fly-belt. Isolated fly foci often occur at apparently open, flat marshes miles from the main river, and if they were overlooked they would provide sources of wet season reinfestation and vitiate the success of eradication. Since no accurate maps of Lawra district existed the whole area had to be surveyed and mapped. Some of the senior fly-recorders were trained in the use of the prismatic compass and chain as well as in the recording of botanical and entomological data. Maps of different sections of the river system were checked and combined by the writer, using a framework made by compass and car speedometer readings. In this way the whole of Lawra district was mapped between 1940 and 1943 and the greater part of Wa has now been covered, the resulting maps being remarkably accurate.

Because of the acute nature of the problem a programme of communal clearing was carried out in Lawra and Wa districts in 1939 and 1940, together with the mass treatment of cases over the northern half of Wa and a strip of country along the Lawra-Tumu boundary. At this time only "protective clearings" were made, which aim at cutting the man-fly contact at places where the main transmission is thought to be taking place, *i.e.*, at village water holes, road-river crossings, etc. Uncleared fly-belt remains along the river above and below this type of clearing which is, in consequence, always subject to invasion by tsetse. It is the usual practice for protective clearings to be ruthless, all vegetation along the river banks being removed (McLetchie, 1945; 1948) but in the present case considerable discrimination was exercised in the clearing, all tall clean-boled trees being spared, with a view to the subsequent incorporation of the work into selective clearing projects. A minimum length of 1,000 yards was aimed at, and although this was not always attained it was often exceeded so that the average for all clearings in Lawra was ¾ mile, and in Wa 1,000 yards.

In Wa district protective clearing has been the only method of tsetse control employed, nearly every village in 700 square miles of the north-western part, at that time the most heavily infected, having its clearing by 1940. In some places in the central zone, where trypanosomiasis had persisted at a high level despite the early work, the clearings were much extended in 1944.

In Lawra district the first communal clearings were made at 34 villages showing the greatest infection. The full programme of tsetse eradication was begun in December, 1940, starting on the largest of the Volta affluents, the Kamba river which drains 600 square miles, approximately half of the district. Mass treatment of the whole district was carried out in 1941 with a repetition over the Kamba area in the same year. Before the Kamba clearings were completed in 1942, operations were extended into the southern part of the district and eventually into the north-western corner. There were numerous small delays due to religious objections to the clearing of sacred groves which are often the very worst centres for the dissemination of infection. The last of these groves were cleared in 1945. By this time 1,100 square miles of country with a population of 90,000 had been freed from *G. palpalis* and *G. tachinoides*. The operation had involved the clearing of 188 miles of permanent fly-belt, measured along the rivers and streams, giving an average of 0.17 linear miles of clearing per square mile of country freed from fly. The total cost with labour at 6d. a day, and including all overheads such as tools, transport, supervision, was £4,500.

The most dramatic is the case of Gbare, a once prosperous little town that was being literally wiped out by trypanosomiasis. Two hundred and ninety three cases were sent to Lawra hospital during the 3 years up to 1939 and the 1931 population of 887 was halved by 1940. The two groves were cleared in 1939 and 1940 and from 1941 to 1946 exactly five cases have come in from Gbare.

The important feature of the 1947 distribution is that it shows clearly that no local foci of infection have persisted along the uncleared Volta. This was already noticeable in 1945 the year clearing was completed, and was as marked in 1946 as in 1947 Nor does any danger seem to arise on the lower reaches of the tributaries where the annual migration brings up a few tsetse. Within the area of eradication the tendency if any is for cases still to show up in the places which were previously heavily infected and in those most recently cleared, such as the Nandom river and the Lawra groves. This is to be expected in view of the short time since the completion of fly control and the prolonged nature of the disease, with patients coming in voluntarily or being brought in only when obvious symptoms develop

The progress of control and the effectiveness of the means employed are shown by separate analyses of events in the three sections of Lawra district, in which eradication was achieved in different stages, and by comparison of these results with those in two areas of protective clearing in Wa and with the unchecked progress of the epidemic in south west Wa where no control measures have been applied. The results are given in Fig 1 and Map 3.

In Lawra eventual trypanosomiasis reductions of 98 5 per cent. 97 per cent. and 96 per cent. for the Kamba, Southern and North-western areas respectively reflect the order in which the work of tsetse eradication was completed. It is noticeable that the Kamba area shows the most consistent rate of decrease followed closely by the curve for South Lawra, whereas in the north western area the initial decline was largely offset by a rise in the number of infections in the following year and further appreciable reduction did not take place until the clearings were extended in 1943. From this point the curve runs parallel with those for the other two sections. The relationship between the efficiency of tsetse control and the ensuing trypanosomiasis reduction is well brought out in the two Wa areas. In the north western area, 500 square miles in extent, clearings averaging 1 000 yards in length at all villages caused a rather irregular decline in the amount of the disease, very different from the decline consequent on tsetse eradication. Moreover the reduction was not progressive. It had reached 81 per cent. by 1945, but showed no further decline. Two hundred square miles in the centre of the district had clearings averaging 830 yards in length at the larger villages only A very irregular curve showed no significant reductions up to 1944 when considerable extension of the clearings brought a decided reduction, measuring 62 per cent. of the original incidence by the end of 1945 Since then however admissions from this

FIGS—1a and b—Effect of Complete Control and of Partial Control of Tsetse on Sleeping Sickness

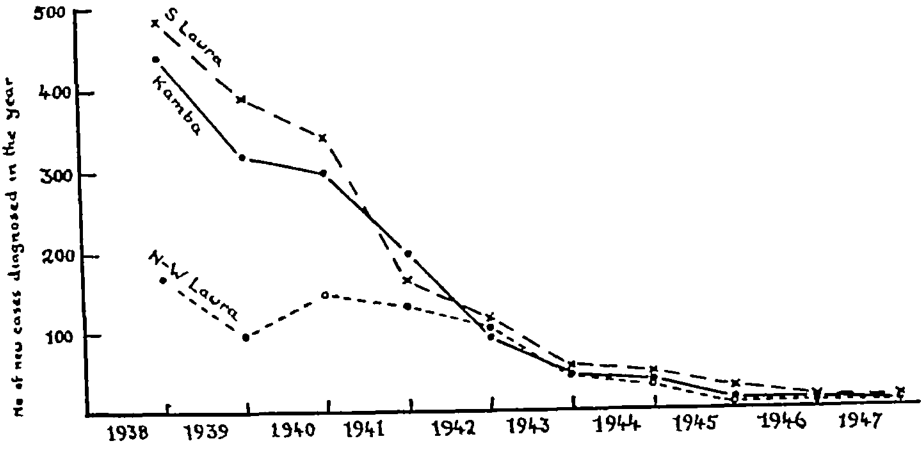


FIG 1a—Tsetse Eradication by Selective Clearing

All areas with local protective clearings, 1939-40, mass treatment, 1939 and 1941
Kamba Area, eradicative clearing, 1940-42
S Lawra, extension of clearings, 1941 eradication, 1942-43
N-W Lawra, extension of clearings, 1943, eradication, 1944-45

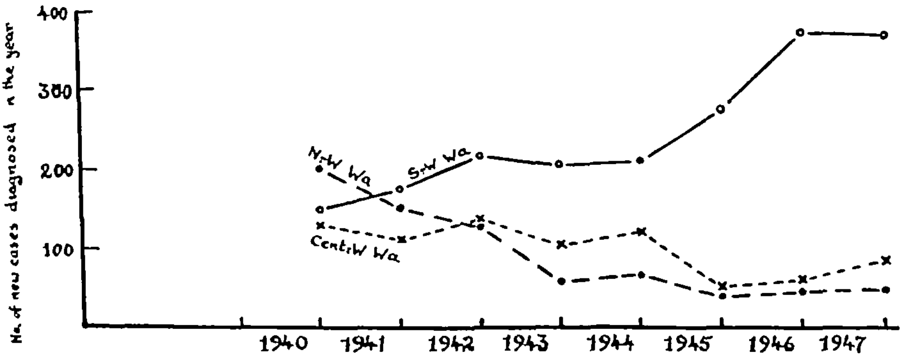


FIG 1b—Partial Tsetse Control by Protective Clearings

Mass treatment in N-W and Central W areas, 1939
N-W Wa, clearings averaging 1,000 yds at most villages 1939-40
Cent.-W Wa, clearings averaging 830 yds at large villages only, 1939-40, extended, 1944
S-W Wa, (control area), no clearing

area have shown a slight increase, so that the 1947 reduction is only 50 per cent The south-western part of Wa district, where neither mass treatment nor clearings were undertaken until 1948, serves as a control area for this series of experiments Two distinct periods of increasing incidence in the disease are separated by a period of stability A new treatment centre to serve this area was opened in 1944, and this certainly accounted in part for the 1945 rise in the number of new cases But more detailed analysis of the data, both over shorter periods and in smaller areas, *e g*, those served by the old Wa hospital only, showed that a real increase, of the nature of 50 per cent, in the amount of trypanosomiasis, took place in south-west Wa between 1944 and 1947 The increase between 1940 and 1942 was 47 per cent, so that a total increase of over 100 per cent has taken place during the period of observation

The relative parts played by mass treatment and clearing in producing these results have been discussed in a previous paper (MORRIS, 1946), so only a brief recapitulation will be given here With incomplete tsetse control the addition of mass treatment appears to increase the rate of reduction, *viz*, the rapid fall in the Lawra areas in 1939 and 1941, but the reduction is not progressive and may not even be stable, *viz*, north-west Lawra and central Wa, where appreciable reduction of the disease took place only after the clearings had been made adequate But with the perfection of entomological control the effects of mass treatment become less apparent, *viz*, the double mass-treatment in the Kamba valley in 1941, when a high degree of tsetse control was established, had less effect than the single mass treatment in South Lawra, where the fly was still numerous The explanation lies in the fact that vector control, by cutting off the supply of parasites before they have reached their hosts, anticipates the work of mass treatment, which affects the parasites only after their establishment in man Thus the more efficient the control of the vector the less will be the effect of mass treatment With complete vector control, *i e*, tsetse eradication, the supply of new infections is cut off and, in the absence of the vector, already infected persons will be of no further danger to public health Mass treatment will now have no effect in furthering the control of the disease, but will, of course, be of humanitarian value in clearing up infected cases

General Inferences

From these experiments, and from other work in the Gold Coast and neighbouring Ivory Coast (MORRIS, 1946, unpublished reports by SAUNDERS and by MORRIS, personal contacts with the French West African Tryps Service), it is possible to draw some general conclusions on the control of trypanosomiasis by clearing

For protective clearings to have the maximum effect they must be expertly sited, *i e*, made at the points where the local people come into the closest and most regular contact with permanent fly-belt Rule of thumb clearings at obvious places such as motor roads and big bridges may be useless if the main fly-man contact is taking place elsewhere The clearings should be as long as possible (at least 880 yards although much higher

control results from 1,000 yards or longer) and should be carried out consistently at all villages over the whole infected area. Such system of protective clearings can bring substantial reductions in sleeping sickness incidence from 50 per cent. to 80 per cent., but the reduction does not progress beyond certain point and is liable to reversal if local conditions become more favourable for transmission (c.f. W in 1946–47 N W Lawra in 1940)

Complete control in severe epidemic areas cannot be attained by protective clearings alone. The addition of discontinuous mass treatment increases the rate of reduction but does not affect the end result, and is not sufficient to bring complete control. Continuous mass treatment during 5 to 8 years together with widely applied clearing campaign (protective and eradicative) has virtually liminated sleeping ickness in the Upper Ivory Coast.

I localized centres of infection such as occur along trade routes very high degree of control can be obtained by the combination of mass treatment and long, well sited protective clearings. (At Bambol ferry on the Volta mile-long protective clearing, which by no means excluded all the tsetse plus single mass treatment, gave higher degree of control than five mass treatments alone in the country immediately to the south.)

Ruthless barrier clearings are no longer employed the results do not justify the expense and labour of their construction and above all of their maintenance. *G palpalis* and *G tachinoides* will freely cross more than mile of perfectly open river bank at any time of year and traverse 5 to 10 miles during the wet season. The labour employed in cutting every tree, including valuable mahoganies, acacias, etc., over barrier clearing mile long by 100 yards wide could make 2 or 3 miles of discriminative or selective clearing It is considered wiser to devote the available resources to the extension of existing clearings along the river thus increasing the distance that flies have to traverse between their permanent habitat and their source f human food. This increases the area protected as well as reducing the amount of man-fly contact.

Eradication of the tsetse results in a rapid and progressive reduction in human trypanosomiasis, with virtual elimination within 5 or 6 years if the area of operations is large enough. Eradication has the additional advantage of simultaneously controlling animal trypanosomiasis and thus allowing a fuller agricultural development of the reclaimed land. Besides its intrinsic value through increasing the prosperity of the community this development has a special value of great importance in that it can contribute towards maintenance of the reclamation.

V THE DANGER OF *G morsitans*.

One of the serious secondary effects of depopulation is that it renders the country suitable for colonization by *G morsitans* a species which is dependent on the larger game animals for its sustenance and consequently can exist only in thinly populated regions where game is abundant.

Surveys by the writer have shown that this species does not occur in country with population density above 15 per square mile and, as sleeping sickness rarely assumed severe proportions with populations below 20 per square mile, *G morsitans* is not involved to any serious extent in the main problem. But when belts of this tsetse traverse or abut on well populated country *G morsitans* presents potential danger to man and ery grave danger to his stock. Because of the virulence of the strains of animal trypanosomiasis carried the presence of this game tsetse constitutes one of the severest handicaps to schemes of cattle improvement and to the development of backward areas.

In consequence, when an invasion of *G morsitans* took place from the Ivory Coast into the Black Volta and central Kamba valleys in 1939 immediate steps to control it were essential, since it brought threat to the general prosperity of large part of the district and particular danger to the chances of settling and dev loping the Kamba valley

FIG 1—A Sacred Grove, where the continuous and intimate contact between tsetse and the villagers, most of whom visit the water hole at one time or another, gives the optimum conditions for the development of epidemic sleeping sickness

FIG 2—Heavy and extensive fly belt on the Volta River, but it is traversed only occasionally and at certain places by natives who do not stay long The chances of flies becoming infected and then re-infecting other people are very small

FIG 3—Travellers waiting for a Volta ferry on a main trade route. At such places the large numbers of natives continually passing, many from infected localities, provide a source of infection for *Glossina* and local though sometimes quite heavy, centres of infection can develop

FIG 4—Occupations such as bathing and washing which bring people regularly to the same point in a fly belt encourage the presence of tsetse, especially *G palpalis,* and set up conditions ideal for the dissemination of infections This shows a typical feeding ground for *G palpalis* with hosts clearly visible from a wide front of closely adjacent fly-belt

FIG. 1.—*Maintenance of the clearings is now taken over by the local Native Authorities.* A Chief watching the weeding out of stable stumps of fly-belt trees after clearing.

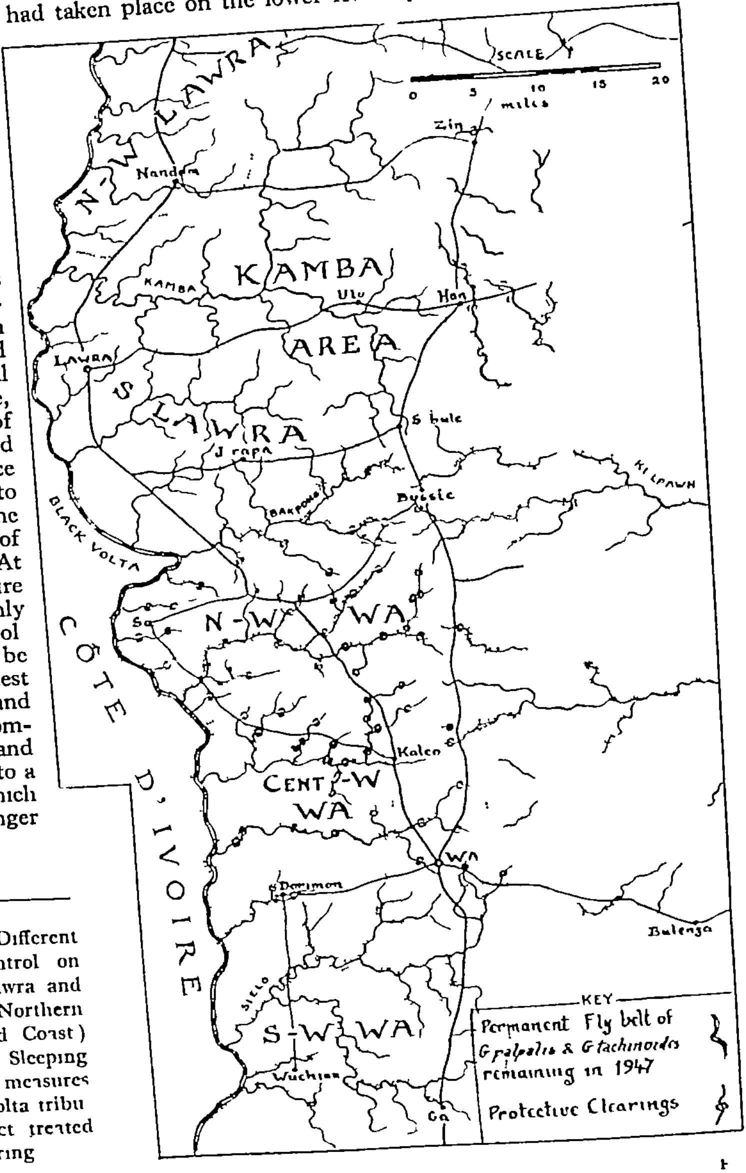
FIG. 2.—*The vegetation change from low evergreen fringing forest to open grass and tall tree association that can be effected by stumping and weeding.* The grass cover along the bank provides good dry season grazing.

FIG. 3.—Maintenance of clearings by farming. Contour ridging, with bananas on the ridges and rice between, has completely reclaimed the banks of cleared stream close to La ra.

FIG. 4.—Maintenance of clearings by farming. A protected clearing on the White Volta that has been extensively farmed by the natives for number of years, limiting the amount of regrowth (mainly *Mimosa* and *S. ebonsa*) to very narrow strip close to the water edge, easily dealt with.

fter the eradication of *G palpalis* and *G tachinoides* A full description of the control of *G morsitans* cannot be given in this paper, it will be the subject of a separate publication and only the briefest summary will be given here Control measures consisted of the disturbance and reduction in numbers of the four species of big game present, roan antelope, hartebeeste, waterbuck and warthog, followed, as far as possible, by the settlement and development of the unpopulated land to prevent the game from returning Despite the fact that between 1939 and 1940 the fly had spread 40 miles up the Kamba and that a 14-fold increase in numbers had taken place on the lower river by 1943, the spread was checked, and by 1947 this species of fly had been completely eradicated from the territory on the British side of the Volta

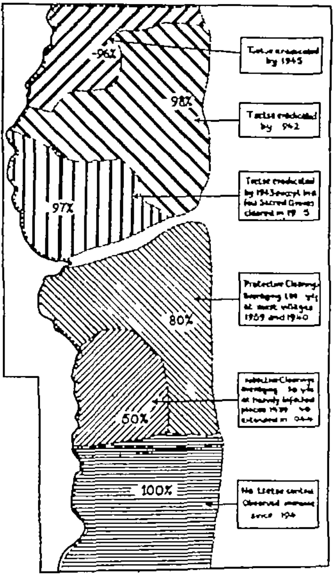
It is of great significance that while control on the central and lower Kamba was still incomplete, from 1943 to 1946, cattle and human beings were present in some numbers and were freely fed on by *G morsitans* This did not prevent the eventual disappearance of this tsetse, a fact that affords final proof of its dependence on wild animals for maintenance and leaves no doubt as to the effectiveness of game reduction as a means of getting rid of the fly At the same time this measure should be regarded as only the first stage in control Eventual control can be obtained with the greatest efficiency, economy and benefit to the local community by development and settlement of the land to a population density at which *G morsitans* can no longer exist



MAP 3—Effect of Different Degrees of Tsetse Control on Sleeping Sickness (Lawra and Wa districts in the Northern Territories of the Gold Coast) Percentage reduction in Sleeping Sickness since control measures began —97% All Volta tributaries in Lawra district treated by selective clearing

VI. Stability in Control.

In combating large-scale outbreaks, whether of disease or of agricultural pests, one is always faced with the difficulty of maintenance. The initial outbreak may be so alarming and costly in life, health or produce that the most expensive measures are employed in reducing it but their continuance, once the reduction has been effected is often hard to justify It is at this point that control measures which are self maintaining or can be incorporated as part of the normal activities of the community or whose cost is less than the estimated losses due to the pest at its reduced level, are usually sought. It is wise to recognize this difficulty from the outset. Thus the biological control of noxious insects and weeds, in which introduced natural enemies effect and maintain a stable control, or cultural control through modifications in the practices of farming or storing may in the long run prove more satisfactory than chemical control by methods of spraying, dusting, etc. which need constant and costly repetition. Likewise in the control of an insect-borne disease the extermination or even reduction in incidence of the vector where practicable has been found more effective and eventually more



economical than the direct attack on the parasite itself by chemotherapy Especially so is this the case in trypanosomiasis where vector control removes at once the danger to man and his stock

It is well recognized that quite apart from the social and physical complexities of its administration over a wide and varied terrain, mass treatment alone is insufficient to stamp out sleeping sickness (WILCOCKS, CORSON and SHEPPARD, 1946, MCLETCHIE, 1948) FAIRBAIRN goes so far as to say that epidemics of *T rhodesiense* infection cannot be controlled merely by treatment (FAIRBAIRN, 1948) VAUCEL holds the view that there is a point below which it is impossible to reduce the incidence by chemotherapy (VAUCEL, 1942) But a high degree of control of serious epidemics has been obtained in both British and French territory in West Africa, the disease being reduced to such a low level that it is no longer a danger to public health Further reduction, however, or even maintenance of the control, by treatment, is expensive, involves a permanent commitment in staff and institutions, and may even be resented by the people The expenses hardly seem justified by the amount of disease present yet the efforts at control cannot be relaxed for fear of further outbreaks This is the impasse into which too great a reliance on chemotherapeutic means leads its advocates

The French have got over the difficulty by turning their Sleeping Sickness Service into a Service of General Hygiene, and using the same teams and organization for the diagnosis and treatment of a number of communicable diseases in the field But they have, from the first, included "prophylaxis agronomique," *i e*, measures for the control of the tsetse, as an integral part of their campaign, and with increased attention to the technique of clearing during the past few years they have now achieved a remarkable success, that of virtually eliminating sleeping sickness from the huge epidemic area within the bend of the Niger* In Nigeria, MCLETCHIE (1948) has shown that efficient protective clearings add greatly to the degree of control effected by mass treatment, and a wide expansion of clearing operations is planned in order to realize the original control plan of treatment plus clearing A most satisfactory example of stable control can be seen in FAIRBAIRN's and MACLEAN's sleeping sickness settlements in Tanganyika, in which a full appreciation of many aspects of the problem has given rise to an essentially simple solution whereby the people,

* Since this was written the author has visited some of these settlements and obtained further information from Dr FAIRBAIRN (Officer in Charge of Trypanosomiasis Research, Tinde) and Dr CALWELL (the present Sleeping Sickness Officer) on the spot There is no doubt that these settlements alone, because of the rapidity with which they were made as soon as each local epidemic was spotted and because of their immediate effect on *G morsitans* and therefore on the incidence of infection, have brought sleeping sickness under a high degree of control throughout Tanganyika Their effectiveness in averting a very serious threat to the Territory can be seen from the Appendix to FAIRBAIRN's 1948 paper An annual incidence of 300 to 500 cases between 1925 and 1927 rose to 3,262 in 1929 but was brought down to the 500 mark by 1936 and is now only just above that level, owing largely to a recent outbreak in Kondoa district which is not yet under control

once they are settled with expert advice, maintain by their own activities a complete control of the tsetse (*G morsitans*) and therefore of trypanosomiasis.

The problem of maintenance assumes especial importance in the huge areas affected by trypanosomiasis in West Africa, so the permanent reclamation of fly infested country has been a constant aim during the present work. For this reason less attention has been given to protective clearings, since it was realized that not only do they afford an incomplete solution to the problem but that their annual maintenance takes up both funds and supervisory staff to such an extent that this would eventually set a limit to the areas that could be covered with a definite personnel. This has happened in Nigeria, where since 1945 the control officers have been almost fully occupied in the maintenance of old protective clearings. (MCLETCHIE, 1948.)

The use of natural land units in the eradication of *G palpalis* and *G tachinoides* offered the possibility of attaining stability through the alteration of the plant communities of the reclaimed river valleys and their subsequent consolidation by settlement and development. Encouragement in this project was given by the example of some of the most prosperous, well populated areas in the north of the Gold Coast in which the people themselves, by their cultivation of every bit of suitable ground on river or swamp and by their search for building poles and firewood, have automatically freed their country from tsetse. So the work of selective clearing was taken a step further to the point of eradicating the fly belt vegetation as well as the fly This was possible only because a definite and limited number of species of trees and shrubs had t be removed. Clearing was followed by a thorough and exacting system of burning and stumping applied on each river system from the headwaters downwards in order to prevent the rapid re-colonization of the clearings by waterborne seeds, roots or branches.

In this way the riverine plant communities in all the Lawra clearings have been completely altered, the closed tree shrub association f the fly-belt being replaced by an open tree-grass association. This alteration has brought a number of incidental benefits quite apart from the disappearance of the tsetse. Under the original closed fly belt association with its almost bare floor the river banks were constantly subject t natural erosion, and this, of course continued during the process of clearing B t soon after stumping was com pleted much of the previously bare bank became covered with a dense mat of grass which is proving a most effective check to erosion as well as helping to smother the re-establishment of shrubs. It should be remembered that on these winding rivers the banks are being continually worn away on the outer bends and built up on the inner a natural occurrence that must not be mistaken

There has been close co-operation in entomological work between the Gold Coast and the French West African Service Trypano, members of which, after study of Selective Clearing at Lawra, has applied the technique with success in the neighbouring Upper Volta country

for artificially induced erosion The fact that such annual maintenance as slashing is unnecessary after proper stumping ensures the consolidation of a semi-permanent grass cover This affords valuable dry-season grazing, of which great advantage is taken by the local cattle Finally, the dense fly-belt was a hide-out for numerous carnivores—lion, leopard and hyaena—which have considerably diminished in numbers since it was cleared, a point much appreciated by the natives

How permanent this change will be can, naturally, not be decided in a few years The fly-belt associations represent the climax vegetation of the riverside and their re-establishment can only be by natural succession, a slow process, probably taking several decades, and therefore easily checked So a precautionary maintenance system has been put into operation, working at present on a 3-year rotation, *i e*, with the district divided into three blocks, each of which is weeded of any new or persistent fly-belt growth every third year It is almost certain that the rotation can be lengthened to 5 years, once the vegetation control is well established The amount of work involved in the weeding is trivial, averaging a requirement of 10 man-days labour per square mile of country every third year, *i e*, just over 3 man-days per annum per square mile Even at such low population densities as 20 per square mile, a turnout of five able-bodied men for 2 days' work every third year presents no difficulties, and the whole maintenance, with the exception of the thinly populated Lower Kamba, has been taken over by the native authorities on a voluntary basis

Settlement and development of land adjacent to the clearings has also been effected by the natives on an entirely voluntary basis, since no material or other inducements could be offered Approximately 1,500 have settled along the Kamba river and its tributaries since they were cleared, and 4,000 acres of new land have been broken for farms Two stock-improvement farms, an agricultural demonstration farm, and a rinderpest immunization camp are being successfully run in places that were swarming with tsetse up to 1942 The settlement is the natural outcome of the advantages of good farm land, water, grazing and timber offered by the river valleys, and has amply justified the full programme of tsetse eradication which took the clearings well beyond the limits of the settlements existing in 1940 Above all the movement is now in the right direction, an advance instead of a retreat, and is resulting in the tsetse fly being replaced by a healthy and more prosperous people

The Lawra experiments have shown that tsetse eradication effects a rapid control of human trypanosomiasis which is, moreover, progressive and will, if applied sufficiently widely, lead to the eventual elimination of the disease Thus it overcomes the first failing of mass treatment, the check at an apparently irreducible minimum The solution to the second difficulty, that of maintenance, has been found in the same experiments By concentrating on the eradication of the habitat of the tsetse as well as getting rid of the fly itself, the clearings can be brought to a point at which they are so nearly permanent that their maintenance can easily be undertaken by the local population, even at such low densities as 20 per square mile This is the lowest density at which sleeping sickness appears to a serious extent in this part of West Africa The system thus overcomes difficulties such as those encountered in N Nigeria by NASH, who requires a minimum population of 70 per square mile for maintenance of his discriminative and protective clearings This involves the complexities and expenses of population shifts and abandonment of ground if areas of lower populations are to be dealt with (NASH, 1948)

The experiments have further demonstrated how the people of their own initiative, will occupy and develop the reclaimed land Indeed, the response and appreciation shown by the natives has been of the greatest encouragement and constant demands are made for an extension of the work. This year (1948) a block of nearly 200 square miles of the Mamprussi district has been cleared with funds supplied by the native administration treasury and a start has been made on the most infected part of the South-West Wa epidemic In both places the natives are eager to make immediate use of the cleared land It is well recognized today that development schemes do not go forward without the willing co-operation of the native population By enlisting this co-operation, through demonstrations of the results that can be achieved by clearing we have on our side one of the most valuable weapons against the tsetse fly the African farmer himself

VII Discussion

The experimental work in the Gold Coast covers areas of sufficient size and variety of terrain, and has been under critical observation for long enough periods for the results to have clarified the rôles of certain techniques in sleeping sickness control. Three principal methods have already been discussed protective clearing for partial control, eradication of the tsetse for complete control, eradication of fly belt vegetation for the consolidation of control. Analysis of the results of the Lawra experiments gives some insight into the epidemiology of the disease and this in turn offers suggestion for a wider planning of attack.

The characteristic of the Lawra epidemic was that the area of high infection did not lie along the Volta River but extended across its tributaries, especially towards their headwaters. A concentration of attack on this main area of infection resulted in the general disappearance of the disease alike on the tributaries and on the uncleared Volta. To arrive at an explanation it is necessary to visualize the picture of the epidemic as a whole There are few places in this part of West Africa where the natives are not bitten more or less frequently by tsetse yet serious trypanosomiasis is confined t quite limited areas. These areas show a marked zonation w th localities of h gh infection surrounded by zones of lighter infection beyond wh ch the disease is of sporadic occurrence only There are often linear extensions, sometimes of quite high infection, along trade routes. This shows to what an extent the transference of infection from region to region is taking place a fact corroborated by evidence from French frontier posts. The main features of this distribution are shown in a map of the Volta Basin epidemic already published (Morris, 1946) which however is of too small a scale to show a narrow strip of light infection that does in fact extend along the middle reaches of the Black Volta, and which appears characteristically along the western edge of Lawra district. It is signi ficant that the heavily infected areas are situated on tributaries of the Volta

Rivers or on their upper reaches The explanation of this distribution must be that the complex of factors necessary for building up high infection rates is very exacting and only occasionally fulfilled The full complex is present in the zones of heavy infection In the lightly infected peripheral and intrusive zones all the factors are not present, and it may be that the disease is maintained there partly by constant renewal of infections from the true epidemic centres This must have been the case along the Black Volta in Lawra district On removal of the main reservoirs of infection (the French had the epidemic well under control on their side also), the local transmission of the disease on the big river was insufficient to maintain even a low endemic and the disease rapidly died out

It is now necessary to examine those factors favourable for the transmission of infection which were present in the true epidemic areas and absent from the Volta side They will be considered under five headings (1) The species of tsetse, (2) the movement of the tsetse, (3) the proximity of the people to the fly-belt, (4) the proportion of the population in contact with tsetse, (5) the continuity of contact

(1) In the Lawra district the most outstanding difference between the Black Volta and the lower parts of its tributaries on the one hand and the upper reaches of these streams on the other, was the scarcity or absence of *G palpalis* in the former habitat and its presence away from the main river It was common to find this species increasing in abundance as one ascended the tributaries until, in certain dense groves, notably at Tizza, it was the only tsetse present *G tachinoides*, on the other hand, is very abundant on the Volta and, although common enough on the side streams and in most of the groves, its incidence in them was only one-half to one-twentieth of that found along the Volta Both species are known to be important vectors of human trypanosomiasis, but there are strong reasons for the belief that *G palpalis* is the more dangerous of the two In every one of the heavily infected areas, and in the majority of those in the Ivory Coast, *G palpalis* is present, occasionally alone, more often with *G tachinoides* Within the Gold Coast in places, where only *G tachinoides* is present, sleeping sickness is absent or occurs at a low incidence It is true that a large tract of Northern Nigeria has trypanosomiasis in pure *G tachinoides* country and that in the Mossi country between the upper Red and White Voltas, outbreaks of considerable intensity developed in the presence of *G tachinoides* alone But these outbreaks developed under very special circumstances which will be referred to presently, and in any case they never showed the extensive areas of high infection that were located in neighbouring territory of the upper Black Volta, where *G palpalis* also was involved

More direct evidence comes from a recent study on the Black Volta in the Lawra and Wa districts which has shown the close association of *G palpalis* with the presence of human settlements With uniformity of habitat and

climate the greatest incidence of *G palpalis* was always found in places where the human population was highest and in closest contact with the fly belt. This species diminished in numbers as the population thinned out and it was altogether absent on unpopulated stretches of river On the Volta tributaries the same rule holds good. *G palpalis* disappears only when the population is so dense that human activities destroy its habitat. This distribution i explained by a marked preference for human and domestic animals as hosts, a preference which is confirmed by observations on the reaction of *G palpalis* to traps (MORRIS and MORRIS, 1949). In contrast, *G tachinoides* seems fairly impartial in host choice with a slight preference for wild animals. *G palpalis* then, acquires special importance as a vector It will as long as it has the opportunity continue to feed on human beings, a habit which gives it greater chances of becoming infected and once it is infected adds to the likelihood of the infection being passed on. *G tachinoides* on the other hand, has less initial chance of infection, and even if it becomes infected it may never bite another person.

(2) It has been seen that *G palpalis*, by choice confines itself to the vicinity of man. It frequently happens in Inland Savanna country that restrictions of environment cause an obligatory association between tsetse and man. In the country we have been dealing with, this restriction reaches its extreme form in the groves in which both species of tsetse because they are confined to a very limited habitat, are likewise confined to a limited choice of hosts. The host happen to be man and his domestic animals since, in many cases, the groves are isolated by farming activities and wood cutting and invariably surround a village water hole. Therefore as long as they occupy a grove both *G palpali* and *G tachinoides* are bound to feed largely on man. In such circumstances *G tachinoides* is equally as important as *G palpalis* a a vector and it is far more dangerous than if it were free to move about in the extensive habitat of the Volta fly belt with an ample choice of hosts.

Intermediate between the Volta and the groves are the tributaries with their narrow fringes of fly-belt by no means continuous and often quit limited in length. The movements of the flies are not so restricted here as in the groves but there is wider range of localities where the people can get bitten during their everyday activities in the bush and at water-holes. Thus the tributaries can be responsible for both immediate and remote infections and, although they do not provide the very intimate association between man and tsetse found in the groves, they are undoubtedly serious sources of infection. Further as one ascends these streams the fly-belt becomes increasingly restricted in extent so that the headwaters often present the extreme conditions realized in the groves.

(3) Proximity of fly belt to human activity hardly exists along the Black Volta but it is a frequent feature in villages on its tributaries. This must be an important factor in the building up of high local epidemics judging from the distribution of the disease and from the fact that so many villages close t tsetse-infested rivers have ceased to exist. The author has found a high degree of inverse correlation between the distances of villages from the nearest fly belt and the proportion of their compound in ruins From various reports on the

Volta epidemic (Gouzien, 1907, Gold Coast, 1925–26, Muraz, 1939) it can be concluded that existence within 1 or 2 miles of a river jeopardized a chance of survival of a community

Proximity can act in two ways The closer a town is to fly-belt the greater is the amount of contact between the inhabitants and the tsetse, not just when the people come to the water-holes but because they cannot avoid encountering the river on so many occasions in their search for firewood and herbs, on their way to and from their farms and neighbouring markets, and so on Extreme cases are villages within the loop of a river, in the angle made by two joining streams, or between two closely parallel streams, where the final result has usually been complete abandonment Admittedly these are not simply cases of proximity but involve the additional complication of a periphery of exposure which may be so lengthened by the configuration of a river that the people can hardly leave their village without crossing it Less extreme, but very dangerous, is the case of hamlets of scattered compounds which may lie on both sides of fly-infested water This is a conspicuous feature among the Lobi of south-west Wa

The second danger of proximity is that the tsetse themselves may come right into villages either because of their wet season habit of ranging, or transported by hosts The survey teams frequently, especially during the wet season, catch *G palpalis* and *G tachinoides* among the houses in villages less than a mile from fly-belt

(4) The proportion of a community liable to be in contact with the tsetse will be influenced by Proximity to the fly-belt, the sources of water, some or all of which may be fly free, and the agricultural and economic habits of the people—the form of the village, whether compact or scattered, the methods of farming and herding, and the pursuits of the people which may lead them to riverside bush The importance of the proportion of contact is twofold Firstly, it influences the probability that infected people in the community will pass on the infection, *e g*, a sick person in a village miles from a fly-belt may never encounter another tsetse during his illness, whereas a case in a village on a river bank will have every chance of infecting more flies Secondly, it regulates the degree of infection that can develop in a community, *e g*, in a village with its water supply entirely within fly-belt all those visiting the water are, at some time or other, exposed to infection, whereas in a village with part of its water supply from fly-free sources, the total infection rate may be quite limited although apparently severe outbreaks occur in those compounds using the dangerous water-holes

This factor is only partly responsible for the concentration of infection on side streams A great part of the upper reaches of the tributaries consist of completely fly-free marshes or open water-courses, but a fly-infested stretch of permanent water may constitute a source of danger not merely to its nearest village but to all that are forced to use it in the dry season Although no parts of the Volta in the Lawra area are fly free, the villages here are too far distant for any but a few of their inhabitants to visit the river at all regularly, and there are often alternative sources of water, fly-free, in marshes, pools, etc These alternative sources occur and are used more especially during the rains, which means that contact with the Volta fly-belt takes place for only part of the year This leads on to the next factor

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in continuity may allow the infection to die out or even prevent its ever appearing, *e.g.*, the control of sleeping sickness among palm cutters in Northern Nigeria (NASH, 1944), and the observed fact that the incidence of sleeping sickness is usually much lower in Volta fishing camps than in neighbouring farming villages despite the much closer contact with *Glossina* in the case of the former The fishing camps are vacated annually during the rains.

The importance of continuity is well brought out by the comparison of conditions in the upstream fly-belts with those along the Volta. The general scarcity of water away from the big river and its main tributaries means that water-holes situated in groves and head-water fly-belt are used constantly by the same villages throughout the year Further the water in the dry season is so limited that large groups of women and children are concentrated for many hours of the day at the one or two available water-holes, waiting to fill their pots, washing their clothes, watering their beasts, and so on. The tsetse assured of this regular source of food, tend also to concentrate at the same spots, not only throughout the day but day after day These conditions would appear to be ideal for initiating infection among the flies and then passing it on to number of people from the same village I this way local epidemics can be quickly built up. Infected tsetse may be present at any time and whether they remain in the same place as in groves, or move about as on water-course they are ready t pass on the infection to travellers and visitors who may come from fly free localities. So the local epidemic, because of the continuity of contact between the people of village and the fly acts as a centre for diffusing the infection more widely This aspect should be contrasted with the fly-belt on the Volta which is used as a source of water for part of the year only and is crossed occasionally by small numbers of people who rarely linger for more than half an hour in the actual fly-belt. Under such circumstances the chances of an initial infection arising among the flies will be very small the chances of a local centre of infection being set up and maintained by the regular presence of infected persons will be rarer still and will be likely to happen only at one or two of the most frequented crossings when the amount of sleeping sickness in the country is very high. Thus the Volta, without permanent villages on its banks, could not be responsible for building up epidemics though it could well help in disseminating infection when the disease is already present. But it is most important to remember that this was not always the case. At the beginning of this century there was an extensive river traffic along the Black Volta and numerous towns and villages were situated quite close to its banks. This was unquestionably the location of the original serious outbreaks of trypanosomiasis and the source from which they spread to the hinterland. The occasional towns still located on the Black and White Voltas were all found to be more or less heavily infected moreover in the south-west of W there are recent Lobi settlements close to the Black Volta where serious outbreaks are threatening

There are other factors influencing the distribution of sleeping sickness, the most important being the density of the human population. Serious epidemics do not appear to occur with a population density below approximately 20 per square mile, although local outbreaks may be found, for example along a well frequented trade route or where an important town is situated right on a fly belt At low population densities contacts between the people of different villages will be so irregular that the introduction of infection will be a rare occurrence and the rapid spread of the disease almost impossible. Nor can there be that continual renewal of infection that must be a feature of epidemics in well populated country where the amount of the disease in circulation and the constant movement of people ensures that, if infection should for any reason die out in a particular village there will surely be a re-introduction sooner or later as long as the disease is present in the country

There is an upper limit in population density for the existence of sleeping sickness—something between 200 and 400 per square mile, at which intensity agricultural and domestic activities have resulted in the automatic clearing of the fly-belt But before this upper limit is reached the habitat of the flies has, for the same reasons, become so restricted that ideal conditions for local epidemics are set up

Population density, therefore, determines whether an epidemic can appear in a region or not, but within the well-populated parts of the epidemic areas known to the writer there does not appear to be a direct relation between the population figure and the intensity of the outbreak The influences of the local population are analysable under one or other of the factors already discussed

One last factor that might be considered of importance is the population density of the tsetse itself A careful study has so far failed to reveal any correlation between the numbers of tsetse as shown by their contact with human observers, which is the relation that counts, and the incidence of sleeping sickness At the same time there are plenty of instances, both in the Lawra area and elsewhere, of serious outbreaks occurring in the presence of very few flies, and absence or rarity of the disease where tsetse are very numerous It so happens that the very factors which limit the numbers of the *G palpalis*-group tsetse are those which favour the building up of epidemic trypanosomiasis Fluctuations in the numbers of tsetse, however, may well be accompanied by corresponding variations in the incidence of trypanosomiasis FAIRBAIRN (1948) shows the apparent relationship between long-term cycles in the numbers of tsetse and sleeping sickness in Tanganyika In the incompletely controlled areas of Wa district, and of North-West Lawra up to 1942, the irregularities in the rates of trypanosomiasis reductions that can be seen in Fig 1 might be attributable to the recent increases in the number of flies that have been such a prominent feature of the Volta river observations (page 174) It is suggested that these phenomena do not represent a crude relationship between the numbers of tsetse and of sleeping sickness cases, but that either the increased fly populations themselves or the conditions responsible for the increase bring tsetse into unusual places where new contacts with man are established, and in this way the opportunities for the transmission of infection are increased

It will be remarked that the discussion has proceeded so far without any reference to those two important factors in transmission, the infectivity of the trypanosome to the fly and the transmissibility of the infection to humans The reason is that although so much work has been done on these subjects there is virtually no information on their operation and importance in living epidemics in the field, and it is quite impossible to assign to either factor values which might explain any of the fluctuations and patterns of the outbreaks under investigation The effect of temperature (of the puparia) on transmissibility shown by BURT (1946) and of the temperature of the adult flies by FAIRBAIRN (1948, and unpublished paper), could hardly influence the location of epidemic outbreaks in the same district, but might, however, be an important factor when comparing regions of such different climates as the high temperature zones of northern Savanna and the comparatively low temperature zones of Forest and Coastal Savanna Indeed, low temperatures might be one of the more decisive factors limiting the amount of sleeping sickness in the Forest and on the Coast, *viz*, KINGHORN, YORK and LLOYD's failure to infect flies or find infected flies at temperatures below 75 5° F in their Luangwa Valley experi-

ments (KINGHORN A., YORK, W and LLOYD, L. 1913). But the picture is by no means as simple as, for example NASH postulates (NASH, 1948) since the effect on male and female flies is markedly different, transmissibility in the male increasing with temperature but in the female fly falling off sharply above 83 F a temperature which is exceeded for many months of the year in northern Savanna. Certainly both infectivity and transmissibility appear to operate against the facile transmission of infection and prevent *T gambiense* sleeping sickness from ever assuming the wildfire proportions which might be expected from the amount of man fly contact taking place throughout the country It is because of this innate difficulty of transmission of the trypanosome that such precise combinations of environmental factors are necessary for serious outbreaks to develop

In conclusion, a brief review of the infected country as a whole takes us a step further towards an understanding of the mechanism of epidemic trypanosomiasis. The key to the problem lies in the most northerly parts of the epidemic In the Mossi country of the upper Red and White Voltas, where the development of high infection rates in the presence of a single vector *G tachinoides* gives a degree of simplification. In this country the conditions closely resembled the extremes found in the Lawra groves, but were of much more widespread occurrence. A 20-inch precipitation falling over a period of 3 months so restricts the amount of available water and also the extent of the flies habitat that the closest possible contact was maintained between the people and the fly almost continuously throughout the year The population mostly agricultural, is high, which further restricted the tsetse's habitat and ensured that wherever a fly infested river existed there would be ample contact between the farmers and the fly in addition t the water-hole contact close t the village. The density of the population and the fact that the Mossi are great travellers, also favoured the spread of the disease In consequence wherever *G tachinoides* was found heavy epidemics developed and the amount of remote infection that ensued caused the disease to be of quite frequent occurrence in fly free villages. The only limit to the extent of this epidemic seemed to be the absolute absence of tsetse a very different state of affairs from that prevailing farther south. The most significant feature of this Upper Volta epidemic was that high rates of infection occurred right up to the northern limit of the range of tsetse. (Map 1 MORRIS, 1946).

This affords a striking contrast t the central nd southerly parts of the Savanna zone from about 12° N to 8° N Despite the presence of an additional vector in *G palpalis* and the fact that the majority of the water-courses are or have been, infested with *Glossina*, outbreaks of sleeping sickness although serious and extensive, have been confined to certain areas only and become less frequent as one goes south. An even greater contrast is to be found in the Forest where *G palpalis* is ubiquitous and several other species also present but sleeping sickness is either absent or present at low rates of endemicity

Again, the information from the Volta Basin map just quoted, and McLetchie's two maps showing the distribution of sleeping sickness and tsetse fly in Nigeria (McLetchie, 1948), is revealing. In the Nigerian distribution the correspondence between the southern limit of the disease and the southern limit of *G. tachinoides* is noticeably close. The same is true in the Gold Coast; with the exception of a localized centre at Ejura, no areas showing 2 per cent infection or over have been found beyond the southern limit of *G. tachinoides*. The interpretation is not that *G. tachinoides* is necessarily responsible for a large amount of transmission, but lies rather in the implications of the presence of this dry savanna tsetse. *G. tachinoides* is a sure indicator of a country where the rainfall is small, the dry season long with low relative humidities, and the vegetation of an open xerophytic character except along the rivers with their evergreen fringing forest—in other words a country in which the habitat of the fly and the water supplies for the people being restricted, brings about that intimacy and regularity of contact that has been seen to be essential for the development of endemic trypanosomiasis.

In the region where these epidemics have been most severe and extensive (approximately 9° 30′ N. — 12° N.), the northern limit of the distribution of *G. palpalis* is being approached and it is this species which is here subject to restrictions in its habitat and therefore of movement; both environment and host preference combine to bring this tsetse into close association with man and make it unquestionably the vector of major importance. *G. tachinoides*, on the other hand, free from the restrictions that force it into association with man in the north of its range, now enjoys an optimum and comparatively extensive environment and plays only a minor rôle in the transmission of trypanosomiasis. But in the Forest region *G. palpalis* is living in a more favourable environment than in northern savanna, it has an extensive habitat in which it can move about freely, and the people themselves are not restricted in their settlements and activities by shortages of water. Thus several of the factors conducive to the development of high rates of infection do not operate in the Forest, and it is quite possible that this is sufficient to account for the limited extent of sleeping sickness in that region.

The influence of the numbers of infected persons in determining whether or not an epidemic will be built up is another factor, verging more on the mathematical, that is in addition to the biological and environmental influences. A favourable combination of factors leads to local outbreaks from which the disease spreads and the numbers of cases increase until eventually transmission of infection is going on in all sorts of places and under conditions where it would normally never occur. Consequently a widespread and heavy outbreak develops that will be checked only by a change in the factors favouring transmission. The change can occur naturally. Depopulation along the Black Volta so reduced the intimacy and continuity of fly-man contact that this locality became a secondary instead of a primary focus of the outbreak. The process might have gone further in the absence of subsequent intervention, and the Lawra epidemic might at length have burned itself out—at the expense of the river valley populations. In other words sleeping sickness is a dynamic and not a static disease and changes can work in either direction. Although conditions in the Forest do not at present favour epidemic sleeping sickness, it must be

ments (KINGHORN A. YORK, W and LLOYD, L., 1913) But the picture I by no means as simple as, for example, NASH postulates (NASH 1948) since the effect on male and female flies is markedly different, transmissibility in the male increasing with temperature but in the female fly falling off sharply above 83 F., a temperature which is exceeded for many months of the year in northern Savanna. Certainly both infectivity and transmissibility appear to operate against the facile transmission of infection and prevent *T gambiense* sleeping sickness from ever assuming the wildfire proportions which might be expected from the amount of man fly contact taking place throughout the country It is because of this innate difficulty of transmission of the trypanosome that such precise combination of environmental factors are necessary for serious out breaks to develop.

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The influence of the numbers of infected persons in determining whether or not an epidemic will be built up is another factor, verging more on the mathematical, that is in addition to the biological and environmental influences A favourable combination of factors leads to local outbreaks from which the disease spreads and the numbers of cases increase until eventually transmission of infection is going on in all sorts of places and under conditions where it would normally never occur Consequently a widespread and heavy outbreak develops that will be checked only by a change in the factors favouring transmission The change can occur naturally Depopulation along the Black Volta so reduced the intimacy and continuity of fly-man contact that this locality became a secondary instead of a primary focus of the outbreak The process might have gone further in the absence of subsequent intervention, and the Lawra epidemic might at length have burned itself out—at the expense of the river valley populations In other words sleeping sickness is a dynamic and not a static disease and changes can work in either direction Although conditions in the Forest do not at present favour epidemic sleeping sickness, it must be

borne in mind that future developments are bound to result in reduction of the natural tsetse habitat in it, and this may well lead to an increase in the intimacy of man-fly contact. Likewise increased traffic will promote the spread of infection. From such changes serious outbreaks might arise One of the duties of the control organization should be to anticipate such possibilities. Moreover by stepping in while conditions are unfavourable for the disease it is often possible to produce the greatest results with the minimum of interference.

VIII The Role of Planning in Control

An understanding of the epidemiology of trypanosomiasis affords a sound background for the planning of large-scale control operations, which, to be successful and, above all, stable must involve a conception of the problem as a whole. A well conceived plan will take account not only of the extent and severity of the disease and the species and particular distribution of the tsetse but also of every aspect of the life of the people and of the country they occupy their past history and most certainly their future development. The population of many parts of Africa is expanding and will continue to do so as long as improvements in health and agriculture are maintained. Campaigns against epidemics and epizootics such as the trypanosomiases need to be planned in terms of future protection as well as of immediate control

As long as sleeping sickness is considered to be the major problem the full resources should be concentrated first of all on eliminating the disease in the true epidemic centres by the most adequate means available. The experience of the writer and that of other workers, indicates that the eradication of the tsetse is necessary to attain this end. The more lightly infected areas should be dealt with when this first stage in the attack is completed. This is a matter of economy as well as of expediency Complete control of the serious outbreaks can be expected to have a marked effect on the incidence of the disease in the lightly infected zones and, from our studies of epidemiology it is evident that the full scale measures that are necessary to reduce the serious epidemic will not be required in areas of low infection. Indeed, the factors may be so delicately balanced that a quite minor interference, judiciously applied, might cause the disappearance of the disease It is in these areas, and in those like the forest where tsetse eradication is at present impossible that one can profitably consider the application, alone or in combination of other measures such as mass treatment or prophylaxis, the continued destruction of flies at important points of contact by traps or insecticides, or even changes in the habits of the people brought about, for example, by the provision of fly free water supplies or the consolidation of scattered hamlets into compact villages so as to reduce the periphery of exposure to the tsetse. Until control on a really widespread scale is established it would be well to check the spread of infection along trade routes. This would not be a difficult matter because of the localized nature f the centres of infection on most of such lines of distribution, and a combination of protective clearings with mass treatment or treatment centres should give a high degree of control.

Although human trypanosomiasis [illegible] dealt with primarily [illegible] the [illegible] epidemiological and economic aspects [illegible] of the [illegible] and [illegible] When it has reached the [illegible] of depopulation the [illegible] infection, [illegible] general welfare and prosperity of the [illegible] when the [illegible] of the [illegible] would be [illegible] In such [illegible] the very [illegible] control would [illegible] be [illegible] the water [illegible] both [illegible] and [illegible]

It [illegible] the river. The [illegible] the water [illegible] rendered [illegible] development [illegible] improvement and [illegible], which are additional [illegible] the reclamation and which can be of value in considering the game. The advantages of reclamation assume increasing importance today when the emphasis is on development and food production and when population pressure is likely to increase the demand for land. Regarded from this viewpoint there should be no question as to the choice of methods for control. A method that can be incorporated into the normal activities of the people has all the advantages of simplicity, permanence and eventual economy over more artificial methods whether of [illegible] or chemotherapy.

Finally, what might be called a theory of keeping [illegible] control reaches the same conclusions. It can be summarized briefly as follows. The simplest and most direct form of control is the attack on the parasite itself within man. But because of inherent weaknesses of technique and difficulties of widespread application, complete control has not been attained in this way, and greater success has been obtained by going a stage further and attacking the tsetse in order to eliminate the parasite before it reaches man. The method of control has itself been taken to various degrees of perfection, the most elementary being to aim at the flies which were most likely to be infected, *e.g.*, by protective clearings around towns, the most advanced being the eradication of the tsetse whether infected or not, in a selected area, *e.g.*, S[illegible]'s block clearings or Nash's Anchau corridor. Each method appears more ruthless than the previous

one and might be called excessive, but just as tsetse control was effective where mass treatment failed, so tsetse eradication is more effective than mere protection. It is logical that the next stage in trypanosomiasis control should be to concentrate on the tsetse's habitat instead of on the tsetse itself and to alter the vegetation type, e.g. by selective clearing and stumping so as to render untenable to the fly the whole of its natural range. This again might be called excessive but its economies have been demonstrated in the case of maintenance and the fact of its ready applicability to any area of serious *sleeping sickness without the com*plexities and expenses of population shifts. Above all the complete control of human and animal trypanosomiasis attained throughout natural land units opens the way for the final stage in the process the occupation and further development of the land by the people themselves.

SUMMARY

Attendances at well established treatment centres give a reliable index of the incidence of *sleeping sickness within the area served, and have been used* for planning control experiments in an epidemic in the Lawra and Wa districts in the north west corner of the Gold Coast and for studying the effects of the measures employed.

Glossina palpalis and *G. tachinoides* are the main vectors of the *T. gambiense* form of sleeping sickness present and also of animal trypanosomiasis. *G. morsitans* occurs only in thinly populated regions and is chiefly concerned with cattle trypanosomiasis.

Sleeping sickness present along the Black Volta river prior to the beginning of the century had by 1938 developed into a serious pandemic covering over 30,000 square miles of the Upper Volta territory.

Depopulation followed in the most heavily infected localities and, by concentrating the population on the uplands between infected river valleys, was *giving rise to serious secondary evils arising from water shortage* and soil erosion. The extension of *G. morsitans* into depopulated areas was also taking place.

A striking feature of the distribution of the disease in Lawra district in 1938 was the relatively small amount of infection along the Black Volta, where fly belt is heavy and continuous, and the concentration of infection on the tributaries and especially their headwaters, where fly belt is lighter and less continuous or even reduced to isolated groves.

Inferences from this distribution led to planning a concentration of attack by tsetse eradication, on the main epidemic area *(the Volta tributaries)* while the Volta fly belt was to be left untouched.

Eradication of *G. palpalis* and *G. tachinoides* was effected by selective clearing, a method involving the removal over each complete river system of only

certain species of trees and shrubs which are essential constituents of the dry-season habitat of these tsetse

Between 1940 and 1945 1,100 square miles of Lawra district were freed from fly at a total cost of £4,500 A population of 90,000 is affected

The permanent tsetse communities disappeared on each stretch of river as soon as clearing was completed The high degree of control obtained has been shown by continuous observation on three of the cleared rivers, where 0 to 9 flies have been caught per year in places where the pre-clearing catches were 2,000 to 7,500 flies per year The catch at a control point on the uncleared Volta was 20,127 flies in 1947

An intrusion of *G morsitans* was brought under complete control by game reduction backed by settlement

In the area of tsetse eradication a 97 per cent reduction in the incidence of sleeping sickness took place between 1938 and 1947

In Wa district systems of protective clearing were applied over two blocks of country and effected reductions of 80 and 50 per cent of pre-clearing incidence of trypanosomiasis, the reduction being proportionate to the number and length of clearings

In south-west Wa, where no control measures had been applied, an increase of over 100 per cent in the number of cases was observed between 1940 and 1947

Tsetse eradication is considered essential for the complete control of epidemic sleeping sickness and has the added advantage of controlling animal trypanosomiasis

A modification of selective clearing leading to the eradication of the fly-belt vegetation has been applied throughout the Lawra area of reclamation and has given such a degree of stability that maintenance can be taken over by the local natives even at such low population densities as 20 per square mile This is important because sleeping sickness does not appear as a serious problem below this density

Full advantage of the reclamation of the river valleys is being taken by the people, about 1,500 having settled voluntarily along the Kamba and over 4,000 acres of new farms having been broken since clearing was finished

Conclusions on the epidemiology of sleeping sickness show that a very exacting combination of factors, bringing the tsetse into close and continuous contact with man, is required for epidemics to arise Without the full set of factors the disease is absent or present at low infection rates only The factors are analysed and discussed

The discussion leads to the conclusion that the most rapid and complete control of sleeping sickness can be obtained by concentrating attack on the true epidemic centres which lie commonly on tributaries of the main Volta rivers

Eradication of the tsetse and fly belt vegetation throughout these natural land units, followed by their fuller development, affords a permanent solution to both human and animal trypanosomiasis and can at the same time be of economic benefit to the community as a whole.

REFERENCES.

BURTT E. (1946). *Ann. trop. Med. Parasit.* 40 18.
FAIRBAIRN H. (1948). *Trop. Dis. Bull.* 45 1.
GOLD COAST (1927) *Report on Sleeping Sickness in the Lawra District Oct. 1924. Ann. Rep. Med. & San. Dept. Gold Coast* 1925–1926 Accra.
GOUZIEN P. (1907). *Maladie du Sommeil dans le haut Senegal et Niger.* (MSS in Headquarters of Service General d'Hygiene Mobile et de Prophylaxie at Bobo-dioulasso.)
KINGHORN A., YORKE, W. & LLOYD L. (1913). *Ann. trop. Med. Parasit.* 7 183.
McLETCHIE, J. L. (1945). *Farm & Forest* 6 69.
——— (1948) *Trans. R. Soc. trop. Med. Hyg.* 41 445.
MORRIS, K. R. S. (1946). *Bull. ent. Res.* 37 201.
——— & MORRIS, M. G. (1949). *Ibid.*, 39.
MURAZ, G. (1939). Rapport d'Inspection et d'Organisation des regions contaminées de Maladie du Sommeil en Afrique Occidentale et Togo. Bobodioulasso, 1939 (*Ann. Med. pharm. colon.* 1940 38 360.)
NASH, T. A. M. (1944). *Bull. ent. Res.* 35 49.
——— (1948) *The Anchau Rural Development & Settlement Scheme.* Col. Office London H.M.S.O.
——— (1948). *Ann. trop. Med. Parasit.* 42, 30.
SAUNDERS G. F. T. (1938). *Ann. Rep. Tryps. Campaign Gold Coast*, 1938.
VAUCEL, M. (1942). *Rev. Sci. Med. Pharm. et Vet. de l'Afrique Française Libre* 1 N. 1 88-100.

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IRON INTAKE IN NORTH-WEST INDIA

BY

H LEHMANN,*
India Command

HYNES showed in 1945 that there existed a widespread mild iron deficiency anaemia in the North-West of India This was quite contrary to expectation, for in Europe a daily intake of 10 or 12 mg is fully adequate for men, and the iron content of the Indian diet was supposed to be four to five times this figure (HYNES, 1945)

The following investigations on the two staple foods of North-West India, wheat and pulses, were undertaken with a view to throw some light on this discrepancy Three lines of approach were chosen (1) a dietary survey, (2) iron analysis, (3) determination of the "available iron" *in vitro* and *in vivo* Of the third part of the programme only the experiments on *in vitro* available food iron were concluded, the work on iron absorption was considered too lengthy a project at the time and the author was detailed to carry out another task in another part of India which was then considered more urgent

* My thanks are due to the D M S in India for permission to publish these results which summarize parts of several detailed reports submitted to G H Q (India) during the years 1944–46, to Major M HYNES, R A M C, for his interest and advice, and to Sergeant F P KAYSER, R A M C, for invaluable support Particularly during the dietary survey I received encouragement and help from so many members of the Army and from civilians, both British and Indian, that it is impossible to acknowledge my debt to them by name

METHODS

Food iron was measured following the principles laid down by HILL (1930) which were developed further by HILL and LEHMANN (1941). The material was dried in a water oven and then ashed. Both wet and dry methods of ashing were used. Dry ashing was found reliable for determinations of whole wheat grains, but not for work on digests, unless a platinum crucible was used. In the case of fluids containing biological material, a large surface of dry ash came in contact with the porcelain crucible and as it was invariably alkaline it attacked the crucible and set free iron which was soluble in acid afterwards. Dry ashing was performed at low temperature and the inorganic residue was boiled in N HCl following MCCANCE and SHIPP (1933). As the temperature of ashing was kept low throughout, the additional manipulations for complete extraction of the iron suggested by MCCANCE, WIDDOWSON and SHACKLETON (1936) could be omitted.

α α'-dipyridyl in excess was added, followed by 4 mol. of sodium acetate for each mol. HCl, and a reducing agent was added in the form of solid grains, usually sodium hydrosulphite or hydroquinine. The different colours thus developed were compared with solutions of known amounts of reduced iron and α α'-dipyridyl which had been standardized by titanium titration.

All reagents used were tested for the presence of iron and were found to be practically iron free. The sodium acetate solutions were boiled and filtered. The HCl stock was a pre war Merck product. Sodium hydrosulphite contained on the average less than 0·02 mg. of iron per gramme.

The availability of the iron was tested in samples of prepared food as it was actually eaten in North-West India. The samples were ground in a mortar and then extracted with 10 vol. of water or HCl for 2 hours at 37° C. The concentration of the HCl chosen was that found in healthy stomach juice i.e. 0·033 N. In some extractions 0·5 per cent. pepsin (BDH) was added.

DIETARY SURVEY

The adult man in North-West India has three meals, morning tea, lunch and supper. He often takes curdled milk between meal times. The morning meal usually consists of sweetened tea with milk only. The more wealthy classes may occasionally eat puri or parotha (small fried cakes) on this occasion as well. Lunch is taken at 9 a.m. and consists of chapattis (unleavened bread) and dhal (pulses) made palatable by the addition of fat and spices and possibly some meat. Supper is consumed at 7 p.m. and is identical with the lunch. The more wealthy may add to this last meal twice or three times a week a dish of halwah (a sweetened dish made from flour).

( ) *Atta and Chapattis.*

Atta is crushed wheat which is mainly eaten in the form of chapattis. It is mixed in a relation of 4:25 to 4:30 with water. No salt or yeast are added. The dough is left at room temperature for 30 to 60 min. and is by then well glutenised, the wheat gluten has given it a sticky coherence. Flat oval discs of different sizes for Mohammedans and Hindus are heated on an iron plate abov fire; they are left for about 30 seconds on each side on the plat , after which they are ready to be eaten.

The water content of atta is 10 per cent, that of chapattis is 36 per cent, thus chapattis contain 71 per cent of atta Table I summarizes some average data on Mohammedan and Hindu chapattis

A Mohammedan eats three chapattis at lunch and three at supper time, a Hindu has, with similarly remarkable consistency, about five on both occasions This is the case whether there are more chapattis available to be eaten or not Usually people cannot eat more Unlike bread, chapattis are not an easily digestible staple food, but can only be eaten as concomitant food with dhal It is noteworthy that "Frontier" chapattis as eaten by some Pathans are much more appetizing and easily digestible than the ordinary Punjabi chapattis They are made with the addition of some sour dough left over from the previous

TABLE I

AVERAGE DATA ON CHAPATTIS

	Mohammedans	Hindus
Size in cm —		
Long axis	23	20
Short axis	21	19
Thickness	0 7	0 5
Weight in grammes	86	51
Moisture in per cent	36	36
Atta content in grammes	61	36

day and therefore leavened These Pathans actually eat up to about 50 per cent more atta in the form of chapattis than the Punjabis The usual intake of atta in the form of chapattis can be summarized as follows —

A Mohammedan eats twice daily three chapattis = 6 × 86 grammes = 516 grammes of chapattis

A Hindu eats twice daily five chapattis = 10 × 51 grammes = 510 grammes of chapattis

510 grammes of chapattis = 360 grammes of atta

Thirty different places, Mohammedan and Hindu alike, were investigated in various areas of North-West India The chapatti consumption was measured by weighing meals as they were served and the average intake of atta in the form of chapattis was found to be 490 grammes = 350 grammes of atta (Table II)

If in addition to chapattis at the main meals, puris and parothas are eaten at breakfast the average atta intake per day rises by another 34 grammes In 17 different places where these cakes were eaten at breakfast the average consumption amounted to 47 5 grammes daily, this corresponds to about 34 grammes of atta (see Table III) Furthermore, if halwah is eaten on the evening of every second day, the atta intake can rise by another 85 grammes One halwah meal contains about 170 grammes of atta

The atta intake can therefore be summed up as follows The usual intake is 350–360 grammes, in the more wealthy strata of the population it can rise to 480 grammes It can be taken for certain that this latter value represents an optimum intake which is only rarely achieved

(b) *Dhal*

Dhal (pulses) is eaten after soaking in water and boiling with salt and condiments, some ghee (boiled butter) and possibly meat are added as well. The volume of dhal is greatly increased during cooking. If 100 grammes of dhal are served as a fairly thickish dish, the volume taken up is 51 ml. Dhal is served in bronze vessels (katturis) or from spoons (chamchas). A big katturi holds 240 ml. comfortably, a small one holds 160 ml. A chamcha holds 120 ml. In 48 out of 53 places the intake per meal was either one large katturi, one and a half small katturi or two chamchas full of dhal. This amounted to 240 ml. of dhal per meal. A North-West Indian eats twice daily 240 ml. of dhal = 480 ml. = 94 grammes of uncooked dhal.

The North West Indian eats his dhal according to volume and within a wide range takes no notice of the consistency of the dish. Often dhal is served more alike in thickness to soup than to porridge; the actual intake of dhal may then fall to 60 to 70 grammes.

Table II.

TOTAL AMOUNT OF CHAPATTIS IN GRAMMES PER ADULT MAN CONSUMED AT 30 DIFFERENT PLACES IN NORTH-WEST INDIA.

Grammes chapatti consumed.	Number of places.
610	1
600	1
540	2
510	3
490	14
480	1
450	4
410	1
350	1
340	1
300	1

Average 490 grammes, corresponding to 350 grammes of atta.

The two main sources of dietary iron in North-West India are atta and dhal and with the various reservations made above it can be taken as a fair assumption that the average intake of these foods is 380 grammes of atta and 94 grammes dhal.

IRON CONTENT OF ATTA AND DHAL

(a) *Atta.*

The North-West Indian flour (atta) is nothing but crushed wheat. It should, therefore, be expected that both the iron content of atta and that of wheat were similar. However the *Indian Health Bulletin* (1941) gives the iron content of wheat as 5·3 mg. per cent. and that of atta as 7·3 mg. per cent. This

difference cannot be due to a different moisture or ash content of wheat and atta, as these values are almost the same for both, 13 and 1 5 per cent for wheat and 12 and 1 8 per cent for atta (*Indian Health Bulletin*, 1941)

ELVEHJEM, HART and SHERMAN (1933) have pointed out that iron estimations in foodstuffs show considerably variable results, and that each value should always represent an average of a number of investigations

TABLE III

ATTA CONSUMPTION PER ADULT MAN PER DAY AT 17 DIFFERENT PLACES IN NORTH-WEST INDIA, WHERE PURIS OR PAROTHAS CONSUMED AT BREAKFAST

Grammes puri or parotha consumed	Number of places
90	1
51	12
36	1
26	2
15	1
Average 47 5 grammes corresponding to about 34 grammes of atta	

TABLE IV

IRON FOUND IN ATTA IN NORTH-WEST INDIA

Sample	Source	Method of ashing	Fe mg per cent
1	Rawalpindi	Dry	3 0
1		Wet	3 0
2	Thelum	Dry	3 0
3	Campbellpore		3 4
4	Sialkot		3 6
4		Wet	3 6
5	Lahore	Dry	3 8
5	"	Wet	3 8
6	Rawalpindi	Dry	4 0
6	"	Wet	4 0
7	Haripur	Dry	4 7
7		Wet	4 4
8	Taxila	Dry	4 8
8		Wet	5 2
9	Rawalpindi	Dry	5 5
		Average	3 98±0 8

In our case the average iron content in nine different samples of atta was found to be 3 98 mg per cent (Table IV) This value agreed exactly with that given by RANGANATHAN (1938), who found an iron content of 3 97 mg per cent in atta, but was lower than that of 5 5 mg per cent reported by GOSWAMI and BASU (1938) However, it differed by 45 per cent from that given in the official bulletin on which the estimates of iron intake had been based hitherto

We enquired from the Food Investigation Laboratory, Kasauli (Punjab), as to what iron content they usually found in atta Major KLEIN, I M S, I A M C the Director of the Laboratory, carried out some special analyses for us and his findings are reported in Table V

It will be seen that in Kasauli somewhat higher iron values for atta were found than had been obtained by us, however, the Kasauli values, with an

average of 5 2 mg per cent. were still 29 per cent. lower than the iron content hitherto assumed for atta.

We now turned our attention to different types of wheat and found the iron content to vary within the same variety and that there was in addition a statistically valid difference (standard error 2 34) in the iron contents of "white" and red wheat (Table VI)

For the purpose of our investigation we assumed for atta an iron content of 4 mg per cent. as correct

TABLE V

IRON FOUND IN TT THE FOOD LABOR TORY KARACLI.

Sample	F mg. per cent.
1	3·43
2	3 90
3	5·93
4	6·10
5	6 43
	Average 5

TABLE VI.

NALYSIS OF WHEAT IN NORTH-WEST INDIA.

Type of wheat.	Number of samples.	Moistur per cent	Ash per cent.	Iron mg per cent.
Red	30	11	1 5	3 94±2 1
White	30	12	1 4	5 18± ·0

(*b*) *Dhal*

The dhal most widely used was *Phaseolus radiatus* and most of our analyses were carried out on that variety Table VII shows that w found an average iron content of 8 mg per cent. for this pulse.

Our results agree well with those of the *Indian Health Bulletin* (1941) which gives the iron content of *Phaseolus radiatus* as 8·4 mg RANGANATHAN (1938) finds an iron content of 10 mg per cent. we differ however considerably from GOSWAMI and BASU (1938), who found only 4 mg per cent. of iron in *Phaseolus radiatu* It will be seen from Table VII that we found a wide range of iron content in the same pulse *this n ght expl in the differences noted* in the literature.

AVAILABLE IRON IN ATTA AND DHAL

It was realized that data on the chemical availability f iron n atta and dhal d d not necessarily give information on their vailability n digestion The availability theory for dietary iron was first advanced by HILL in 1930 and was carried to rigid extremes by ELVEHJEM, HART and SHERMAN (1933) The unavoidable eaction set in when HAHN and WHIPPLE (1938) demonstrated that only ne-th rd of the chemicall a ailable food ron was a ailable t the

organism *in vivo*, and further, that physiological availability decreased with the increasing dosage of the chemically available iron given It is now generally assumed that chemical availability of iron has little relation to its dietary value

It may be suggested that considerations of LEHMANN and POLLAK (1942), on the availability of calcium in food, may have a bearing on this problem In the case of calcium, it was shown that sparingly soluble calcium salts formed more soluble compounds with amino-acids It was suggested that chemically almost unavailable calcium salts should become physiologically available, once they were ingested in the presence of protein which was forming amino-acids in the intestinal tract This theory was proved to be correct for men by MCCANCE, WIDDOWSON and LEHMANN (1942), and HALL and LEHMANN (1944) It was thus clear that in the present investigation availability of iron *in vivo*

TABLE VII

IRON FOUND IN DHAL (*Phaseolus radiatus*) IN NORTH-WEST INDIA

Sample	Method of ashing	Fe mg per cent
1	Dry	6 3
2		6 9
3	Wet	7 45
4		7 5
5		8 3
6	Dry	8 6
7	,,	11 4
	Average	8 06±1 7

TABLE VIII

In vitro "AVAILABILITY" OF IRON FROM CHAPATTIS AND COOKED DHAL

Extracted for 2 hours at 37° C with 10 vol of	Percentage of total iron soluble	
	Chapattis	Cooked dhal
Water	10	6
0 033 *N* HCl	28	19 5
0 033 *N* HCl + 0 5 per cent pepsin	42	39

could certainly not be judged from its chemical availability in the test tube and we arranged our experiments to imitate conditions as they may be found in the stomach Hydrochloric acid could be expected to form soluble ferri-chloride from other sparingly soluble iron compounds Reducing compounds in the food could be expected to form the physiologically available ferrous compounds Pepsin by breaking up physically the structure of atta and dhal would help in making sparingly soluble iron compounds of the food available for both, the action of HCl and that of reducing agents Furthermore, pepsin should increase the availability of the iron by producing sulphydril groups from the protein—which by themselves increase the iron solubility (LEHMANN and POLLAK, 1942) We incubated, therefore, chapattis and cooked dhal with HCl and pepsin No attempt was made to divide the soluble iron

into its ferric and ferrous fractions, but we found that the great majority of soluble iron in our digests was in the reduced state

Table VIII summarizes our results, and it can be seen that chapattis and *cooked dhal contain only little iron* soluble in water but that 40 per cent. of the total iron become soluble when these foods are incubated with HCl and pepsin.

CONCLUSIONS.

The iron intake from 360 grammes of atta and 94 grammes of dhal per day can be calculated on the basis of the above investigations to be 22 mill grammes. This figure rises to about 27 mg if amongst the wealthy the diet is complemented by puris, parothas and halwahs. There may be an additional iron intake of 2 to 4 mg from meat potatoes and rice. These foods are not regularly eaten in North-West India, and particularly as regards meat, any estimate would be extremely difficult to arrive at. The Hindus are part or total vegetarians, Mohammedans are allowed to eat all meat except pork, but amongst them only the very rich can be expected to eat regularly appreciable amounts which would allow to postulate a daily intake of more than 0 5 to 1 mg of iron from that source. Possible errors in assessing the iron intake without taking account of meat, potatoes and rice are counteracted by our generous assessment of the dhal intake at 94 grammes a day It is more than likely that this figure which is only applicable when the dhal is eaten in a porridge-like consistency has to be reduced by 15 to 30 per cent. whenever it is consumed as a more fluid food which is often the case. Also whenever rice and potatoes are eaten the daily intake of atta and dhal falls correspondingly

Although a daily intake of 22 mg of iron represents a very much lower figure than the ones usually assumed for North West India, it is yet about *double the amount said to be sufficient to prevent iron deficiency anaemia.* Taking in account even the wide distribution of hookworm disease in North West India, it is unlikely that the blood loss due to hookworms could account for 10 to 12 mg iron a day HYNES (1945) has actually shown that there is no *obvious correlation between iron deficiency anaemia and hookworm load*, and it has been suggested that much of the iron lost in the small intestines in ancylostomiasis is reabsorbed before it can leave the body It was the fact that the iron deficiency anaemia was found in a population which was consuming *twice as much iron as* was thought necessary to prevent this deficiency disease, and which also responded well to ferrous sulphate therapy in its *haemoglobin* formation (HYNES, 1945), which led us to consider whether all the food iron was really available to the body on ingestion. Table IX *shows how with an iron* intake of 22 mg if atta is only eaten in the form of chapattis, and of about 27 mg if the diet is complemented by puris, parothas and halwahs, the tentative assumption can be made that with a healthy digestion the physiologically available dietary iron may vary between 9 and 11 *mg* a day

There are many factors which might influence this iron intake adversely It is only necessary to remember how precariously the food budget is balanced amongst a large proportion of the population of North-West India, how chronic malaria, which is endemic, lowers the appetite, how chronic subacute dysentery, which is also endemic, through intestinal hurry, prevents a full utilization of ingested food We found both a deficiency of stomach HCl and of pepsin production in a large proportion of cases of anaemia with a history of dysentery or of helminth infestation (LEHMANN and KAYSER, 1948) The role of achlorhydria in causing iron deficiency anaemia has been emphasized as well as doubted FOWLER and BARER (1937) have found the iron absorption in achlorhydric persons to be much lower than in normal subjects, and LEITNER (1948), in summing up the evidence, concludes that absence of anaemia in a number of cases of achlorhydria merely shows that lack of stomach acid is not an essential but only an important cause of anaemia

TABLE IX

AVAILABLE IRON FROM 24 HOURS' INTAKE

Extracted with.	510 grammes chapattis, 480 ml dhal, total Fe 22 mg	510 grammes chapattis, 47 5 grammes puris and parothas, halwah corresponding to 85 grammes atta, 480 ml dhal, total Fe 27 mg
Water	1 9	2 3
HCl	5 3	6 5
HCl + pepsin	8 8	10 6

Any lowering of the marginal available iron intake of 9 to 11 mg a day, or any incapacity of digesting it fully, could be expected to result in an iron absorption well below the necessary minimum

Our results may go some way in explaining why an iron deficiency anaemia amenable to iron therapy has been found so widely distributed by HYNES (1945), in North-West India

SUMMARY

1 The iron intake in North-West India from wheat and pulses has been assessed by dietary survey and iron analyses It amounts to 22 to 27 mg per day for male adults

2 Only 40 per cent of the iron from chapattis (unleavened bread) and cooked dhal (pulses) are soluble on extraction with HCl and pepsin

3. The possible bearing of these findings is discussed on the widespread iron deficiency anaemia of North-West India described by Hynes in 1945.

REFERENCES

Elvehjem, C. A., Hart, E. B. & Sherman, H. C. (1933). *J biol Chem* **103**, 61

Fowler, W. M. & Barer, A. P. (1937). *Amer J med. Sci.*, **194** 625

Goswami, M. & Basu, K. L. (1938) *Indian J med Res.*, **26**, 883

Hahn, P. F. & Whipple, G. H. (1938). *J exp Med.*, **67** 259

Hall, T. C. & Lehmann, H. (1944). *Biochem. J* **38** 117

Hill, R. (1930). *Proc roy Soc B* **107** 205.

——— & Lehmann, H. (1941) *Biochem. J* **35**, 1190

Hynes, M. (1945) *Report on Investigations on Anaemia in the Indian Soldier* New Delhi Government of India Press.

Indian Health Bulletin. (1941). New Delhi Government of India Press.

Lehmann, H. & Kayser, F. P. (1949). *Trans R. Soc trop Med. Hyg* **43** 209

——— & Pollak, L. (1942). *Biochem J* **36** 672.

Leitner, S. J. (1948) *Acta med scand.*, **130** 68.

McCance, R. A. & Shipp, H. L. (1933). *Spec Rep Med. Res. Coun. Lond* No 187

——— Widdowson, E. M. & Lehmann, H. (1942) *Biochem J* **36** 633

——— ——— & Shackleton, L. R. B. (1936). *Spec Rep. Med. Res Coun Lond* No **213**.

Ranganathan, S. (1938). *Indian J med Res.*, **25** 677

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STOMACH FUNCTION IN POST-DYSENTERIC DEBILITY *

BY

H LEHMANN

AND

F P KAYSER

(*India Command*)

In the course of an investigation of gastro-intestinal function in cases of mia it was seen that those with a history of dysentery had almost always npaired stomach function This was considered of interest as it might light on the causes of post-dysenteric debility, and particularly on the ia often developing after a period of repeated dysentery attacks

All the patients studied were seen in the Rawalpindi Military Hospital for n Troops There were so many cases of anaemia that it was possible to ut subjects with a fairly clear-cut previous history Three groups were gated, patients with a history of chronic malaria as the probable cause r anaemia, those with a history of advanced helminthic infestation, and a group of soldiers who had suffered from chronic dysentery in the past as possible it was ascertained that the dysentery had been of the bacillary

* s communication is a résumé of work on stomach function in anaemia carried e No 1 Detachment, General Headquarters (India) Anaemia Investigation Team A full account of this work was to appear in an India Government Blue Book on a" As the publication of this Blue Book is greatly delayed, permission has en by the Director of Medical Services in India to publish this short article, for should like to record our thanks We are grateful to Major M HYNES, R A M C, iterest and to Mr DILWALI for statistical analysis

TABLE I

ASSOCIATION OF STOMACH DYSFUNCTION WITH A HISTORY OF DYSENTERY

41 Indian soldiers suffering from anaemia						
Previous history	Number of cases	Average haemoglobin, g %	HCl		Pepsin	
			Normal	Deficient	Normal	Deficient
Malaria	13	9 5	12	1	13	0
Worm infestation	11	7 3	6	5	7	4
Dysentery	17	9 1	6	11	5	12
12 Indian soldiers after recovery from anaemia.						
Dysentery	12	13 8	4	8	4	8

TABLE II

STOMACH FUNCTION AND PREVIOUS HISTORY IN 41 ANAEMIC INDIAN SOLDIERS

Previous history	Number of cases	HCl and pepsin production, both normal
Malaria	13	12
Worm infestation	11	4
Dysentery	17	3

TABLE III.

CHANGE IN STOMACH FUNCTION IN ACUTE BACILLARY DYSENTERY
(11 DYSENTERY PATIENTS AND NINE CONTROLS).

11 patients admitted to hospital with bacillary dysentery				
	Histamine-resistant achlorhydria.	Achlorhydria responding to histamine.	Hypochlorhydria (never > 12 ml. N 0 1 HCl %)	Normal.
On admission	0	1	1	9
After 7 days	1	4	3	3
Nine controls treated with fluid diet and sulphaguanidine.				
On admission	0	1	0	8
After 7 days	0	0	1	8

impairment he was the patient shown in Table III to have suffered from a histamine resistant achlorhydria 1 week after he was admitted to hospital with a bacillary dysentery He was still showing a histamine resistant achlorhydria after 3 months It is possible that cases of post-dysenteric debility which, as shown in Table I, suffered to a great extent from stomach dysfunction may have had a similar history

One possible explanation of the effect of dysentery infection on stomach function was that dysentery bacilli interfered either with the production by the intestinal fauna of factors essential to stomach function, or with their absorption A possible factor seemed nicotinic acid However, treatment with nicotinic acid of a few cases admitted to hospital with dysentery did not seem to prevent the appearance of stomach dysfunction A continuation with J WALTERS and R J ROSSITER of this line of investigation on patients suffering from achlorhydria after extreme starvation, made it likely that the factor involved was riboflavin Riboflavin had a restoring effect on stomach function within 1 to 2 weeks in 15 out of 17 such subjects As it might be expected, liver extract also had a beneficial effect A summary of these experiments is given in Table IV Full details of this work can be found in a Government of India Blue Book on the "Marasmus Syndrome" (WALTERS, ROSSITER and LEHMANN, 1947, see also LEHMANN, ROSSITER and WALTERS, 1947) It may be noted that glucose oxidation is now considered the underlying mechanism of acid formation by the stomach mucosa (CONWAY and BRADY, 1947, DAVIES, LONGMUIR and CRANE, 1947) and riboflavin is, of course, a catalyst of glucose oxidation

TABLE IV

TESTMEAL FINDINGS IN STARVATION ACHLORHYDRIA

RESULTS OF 14 DAYS TREATMENT OF 32 CASES

Treatment	Number of cases	Better	Worse	No change
Nicotinic acid	8	2	3	3
Riboflavine	17	15	0	2
Liver extract	3	3	0	0
Nicot ac. + liv extr	1	1	0	0
Riboflav + liv extr	2	2	0	0
Nicot ac + riboflav	1	1	0	0

SUMMARY

(1) In Indian soldiers suffering from post-dysenteric and post-malarial anaemia there was a significantly higher incidence of achlorhydria and apepsia in those with a history of dysentery Stomach dysfunction was also frequent in cases with a history of helminth infestation, but the number of patients was not large enough to allow a statistic interpretation

(2) Of 12 Indian soldiers who had recovered from a post dysenteric anaemia, eight were still suffering from stomach dysfunction.

(3) Of 11 Indian soldiers admitted to hospital with bacillary dysentery nine had a normal stomach function on the first day but only three were normal on the seventh day Of the eight patients with stomach dysfunction after 7 days seven were normal again after 2 months one who had acquired a histamine resistant achlorhydria was still showing this abnormality after 3 months.

(4) In nine healthy Indian soldiers no deterioration of stomach function was produced by a 7 days treatment with sulphaguanidine and fluid diet.

(5) Nicotinic acid had no beneficial effect on the development of stomach dysfunction in bacillary dysentery when tested in a few cases, but riboflavin seemed to influence beneficially achlorhydria in starvation debility

REFERENCES

COLE, S. W (1944). *Practical Physiological Chemistry*, 9th ed. Cambridge Heffer
CONWAY E. J & BRADY T (1947). *Nature* 159 137
DAVIES, R. E., LONGMUIR, N M. & CRANE, E. E (1947) *Ibid* 159 469
LEHMANN, H., KATER, F P & SIMPSON J W (1946). *Ibid.*, 157 194
——— ROSSITER, R. J & WALTERS, J H (1947) *J Physiol* 106, 24
WALTERS, J H ROSSITER, R. J & LEHMANN, H (1946). *Investigations on the Marasmus Syndrome in the Indian Soldier* Lahore Government Press.

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DIPHTHERIA IN AFRICAN NATIVES IN THE TRANSKEI, SOUTH AFRICA *

BY

N S TURNBULL, M B, CH B, D P H, D T M, D T H,
Assistant Health Officer, Umtata

Diphtheria has hitherto been considered to be rare in African natives GELFAND, in his book "The Sick African," which mainly concerns Rhodesian natives, expresses this view In the writer's previous experience in Nigeria, diphtheria was practically never seen among the native people there For some years now, in the Transkei in South Africa, small outbreaks rarely amounting to more than a dozen cases have been observed The occurrence of epidemics, involving over 100 cases, is, therefore, considered worthy of record The two outbreaks described in this article occurred practically simultaneously in two separate Magisterial Districts in the Transkeian Territories of the Cape Province of South Africa between February and May, 1948

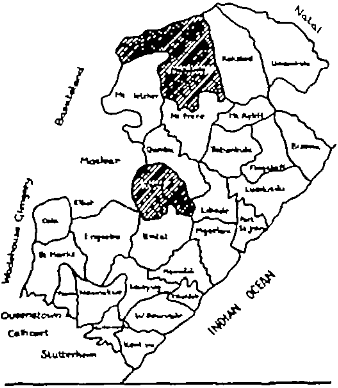
The Transkeian Territories are situated in the north-eastern part of the Cape Province The two Magisterial Districts in which the epidemics occurred are Matatiele and Tsolo (Map) The land in these Territories rises steeply from the sea in three plateaux or step-like formations to the Drakensberg Mountains, which form the western boundary with Basutoland Tsolo lies on the secondary plateau 3,150 feet above sea level, while Matatiele is on the tertiary plateau 1,450 feet higher In both districts the ground is broken and hilly, especially towards the west, where the ground rises sharply and is intersected by deep, rugged, cleft-like ravines

OUTBREAK AT MATATIELE

The capital town of Matatiele lies towards the centre of the district, and from thence roads radiate eastwards through the small village of Cedarville to Kokstad, southwards towards Mount Fletcher and to the north-west through a pass in the Drakensberg Mountains into Basutoland These are the only

* I am indebted to the Secretary for Health for permission to publish this article as well as to Doctors GRAHAM, MEARS and DICKSON for their assistance in producing details of their cases Thanks are also due in particular to the Municipal Council of Matatiele for their loyal support and material assistance in establishing the emergency hospital at Matatiele

main roads in the District and, as most of the cases occurred off the beaten track, horses were frequently required in the search for cases and in follow up inoculations. Immediately south of the town there i a range of hills around the end of which t the west, the road winds for 30 miles over broken country to reach Lupindo s Location, the worst affected in this outbreak. This meant that most of the patients had to be brought into hospital a distance f 30 miles,



Map of the Transkeian Territories 1948

part from arable distance by horse, sledge r in the case of children on their mother backs before they reached the road Matatiele is also the ra l head f a line through Pietermaritzburg to Durban Train are not fast in thi part of the country and it took specimens, at the very least, 16 to 24 hours to reach the laboratory n Durban, some 230 miles away Supplies of antidiphtheritic serum were sent from the South African Institute of Medical Research in Johannesburg by air to Durban n about 3 hours and thereafter had to be sent by rail to Matatiele

Being 4,600 feet above sea level near the foot hills f the Drakensberg it

was expected that cold weather would be experienced earlier in Matatiele than in other parts of the Transkei Actually, the weather throughout April was fine and warm, with little rain, and the first frosts came early in May Together with other parts of the Union, Matatiele experienced drought in the three preceding years, but good rains fell in the summer of 1947–48

Social Structure of the People—Matatiele District is mainly composed of native reserves, with a few European farms to the west of the town and stretching eastwards along the railway line beyond Cedarville The native peoples inhabiting the reserves are mainly Hlubis, with a slight mixture of Pondomises and Hlangwinis In the north-west around Queen's Mercy there is a district colony of Basutos who have crossed from Basutoland to settle in a fertile valley there The cases of diphtheria occurred among Hlubis, the Basutos remained unaffected In the reserves the Hlubis live in circular thatched huts separated from one another One hut acts as cooking, feeding, and sleeping accommodation while in another the mealies are stored Furniture, as such, does not exist, and the inmates sleep on the floor wrapped in a blanket Sometimes one blanket covers as many as three children Three or four huts house a family, and a group of families, comprising 20 to 30 huts, forms a kraal Kraals are separate and distinct, and are usually built on the high ground near the ridge The cattle graze and the crops are grown on the lower slopes towards the valley A group of kraals forms a location which is administered by a headman (the equivalent of a native chief in other parts of Africa), responsible to the local magistrate, who is also the Native Commissioner These headmen bear no relationship to one another, and so each location forms the equivalent of a local clan The location bears the name of a former head (*e g* , Lupindo), and the present headman is usually a descendant of the original, though not necessarily in a direct line Christianity has made little impression on these people in the locations The various missions have provided schools but the mass of the people still adhere to their anamistic beliefs This is most noticeable in any adversity such as sickness This is believed to be caused by a spell cast upon the place where the patient falls sick The " treatment " is to remove the patient from the bewitched area and call in the witch doctor to exorcize the spell The ease with which infectious diseases can spread among these people can, therefore, be readily understood

Occurrence of the Outbreak—The first case was seen by Dr GRAHAM, the District Surgeon, Matatiele, on 2nd March, 1948, in the Municipal Location, and it was quickly followed by others from Lupindo's Location As there were then no isolation facilities, and under the belief that widespread epidemics of diphtheria do not occur among natives, they were returned to their homes after receiving one injection of A D S By 19th March some 20 cases had been seen and, as the Deputy Chief Health Officer, East London, visited Matatiele on that date, arrangements were made to open an emergency isolation hospital for these cases in an old military camp there The epidemic progressed towards its

and age and size of the patient. The dose was reassessed daily and in special cases twice daily Thus a child with laryngeal diphtheria and general toxic condition might have received up to 120,000 units at a single dose. An adult with single patch of membrane and slight toxic condition would still receive the initial basic dose. Experience in this epidemic proved the efficacy of high doses of serum given early in the disease. Improvement in some cases was noted within a few hours. On the other hand, too prolonged treatment with serum was found to be detrimental and some of the patients, after prolonged treatment, showed a rapid improvement when treatment was discontinued. Only one case showed a serum rash which faded within 24 hours. There were no other adverse results from the use of serum.

Penicillin Treatment.—Analysis of the results of throat swabs showed that a large proportion had an associated streptococcal infection. Local doctor stated that throughout the summer they had seen an abnormal number of sore throats. This was borne out by the extremely large proportion of enlarged tonsils seen among the patients admitted t hospital Many of the patients still had enlarged tonsils after recovery from their attack of diphtheria The streptococci were in some instances proved by the laboratory to be *haemolyticus* The infection was therefore, a combination of *Corynebacterium diphtheriae* and *Streptococcus haemolyticus* and the use of penicillin in the treatment was therefore justified. Practically all the patients suffering from diphtheria had both serum and penicillin so there was no evidence of the separate effects of each. Suspects with tonsillitis were given 2,000 units A.D S as a prophylactic in view of the fact that they had to be admitted with other suspects where the diagnosis was still awaiting the result of a throat swab.

Bacteriological.—Early in the epidemic it was appreciated that t would have been an advantage to have a bacteriologist working on the spot and an effort was made to obtain one. Owing to staff shortages at the laboratories, and the presence of poliomyelitis in other parts of the country at the time this effort proved fruitless, and swabs had to be sent all the way to Durban. This is to be regretted as some interesting data might have been revealed. Regarding the swabs, it was found in the interval necessary for the swabs t reach the laboratory that wet " sw bs and Loeffler slopes became so overgrown with commensals as to be useless. Carefully taken dry swabs were most satisfactory In the epidemic, owing to the difficulty in sending swabs, these were used mainly by the District Surgeon as a check on the diagnosis of doubtful cases. Those clinically diphtheria were accepted as such confirmation was sought on only three of these, all of which were positive. No swabs were taken prior to discharge of patients but a total f 72 were taken of which 25 were positive.

The *C diphtheriae* were typed and found to be *mitis* The nfection was, therefore, of a mild type occurring in a non-immune population with an associated streptococcal infection. This was borne out by the clinical findings. In the absence of exhaustive bacteriological in estigation it was impossible to arrive

at a carrier rate but, in this epidemic, one imagines that it may have been high Why, therefore, should the epidemic be so widespread in Lupindo's Location and, with the constant transfer of patients to other locations, should the secondary outbreak be confined to only two other locations?

Emergency Hospital—It was fortunate that the old military camp was so readily available for conversion into an emergency hospital There were 40 beds available for natives and four for Europeans The staff consisted of two European Sisters, one Nun, two Red Cross workers, three native nurses and two medical aids, plus the domestic assistants All did extremely good service A total of 194 patients passed through their hands in the 2½ months that the hospital was open There were 142 cases of diphtheria and 52 suspects These figures do not include the large number of suspect sore throats seen by the District Surgeon at his surgery and treated as out-patients as, in the later stages, after propaganda, the natives were found to come forward more readily with their sore throats

Immunization—It was understood early in the epidemic that passive immunity of close contacts was to be aimed at, with active immunity of the groups less exposed to infection The District Surgeon, therefore, undertook the immunization of European and coloured children with A P T The inaccessibility of the homes of some of the native patients, plus the rate at which cases were discovered and the initial inability to assess the amount of serum required (*a*) for treatment, and (*b*) for immunization made the early immunization of contacts both difficult and irregular It was not until the middle of April that these difficulties were overcome, and it was then considered essential to immunize as many school children and pre-school children as possible with A D S only Accordingly, systematic drives were organized on all the schools in the affected locations, and throughout the epidemic a total of 8,967 of these immunizations were given The difficulty of finding the contacts in the broken country with poor communications, and often meagre information as to locality coupled with the fact that patients had frequently been moved from their original homes, was considerable It was essential to have someone available for this work who was thoroughly conversant with the native of these parts and the country Only two such people, a temporary typhus inspector and a native medical aid, were really successful Other workers imported into the area wasted a considerable amount of time often searching fruitlessly over large tracts of difficult country Once the epidemic was established, the headmen were very co-operative and assisted greatly by providing guides and, once they became accustomed to the routine, gave more accurate information than in the early stages

Outbreak at Tsolo

The District of Tsolo lies on the secondary plateau of the Transkei, some 60 miles from the sea The main road from Umtata to Kokstad traverses the

Bacteriological.—There was considerable difficulty in getting swabs over the long distance to East London with the 3-day per week train service. The bacteriologist frequently reported that Loeffler tubes were contaminated by commensals, or that ordinary throat swabs arrived dry with no resultant growth on culture. Nevertheless, the pathologist at East London was able to report on 27th May 1948, that of the swabs received 18 were found to be of the *mitis* strain as they fermented glucose and dextrose but produced no fermentation with saccharose and starch. Morphologically they were also *mitis* by virtue of the presence of numerous metachromatic granules. There was no mention in this report of a co-existing organism which might account for the rapid spread of th infection at this particula time and which was susceptible to penicillin. Several of the local practitioners, however have reported that throughout the summer they had observed an abnormal number of sore throats presumably streptococcal. It is probable, therefore that the infection was similar to that in Matatiele *i.e.* a combined infection of a *mitis* strain with an associated predisposition due to a streptococcal infection.

CONCLUSIONS

These two epidemics of diphtheria demonstr te that, n certain circumstances, widespread epidemics can occur among African natives. Admittedly the Transkei is not a tropical area and the drought of the past 3 years may be a contributing factor. The epidemics on the whole show no striking differences from those in other parts of the world. Since the middle of June however sporadic cases have continued to appear in both those districts as well as in other parts of the Transkei. It is feared, therefore that diphtheria has come to stay among the African peoples of these territories, and the result of infection by the *intermedius* o *gravis* strains may be left to the imagination. Accordingly it is considered necessary now to undertake a systematic immunization campaign of all children with A.P.T. It is difficult in these areas however to obtain contact with the pre-school children nd the many children engaged in herding who do not go to school.

SUMMARY

1. Two epidemics of diphtheria occurring n Bantu natives of the Transkei n South Africa are described.

2. Difficulties encountered in the nvestig tions -particula ly bacteriological.

3. This is a new occurrence among the Bantu and its future extension may be serious.

4. Further immunization is advocated.

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OBSERVATIONS ON THE ISOLATED GUT OF THE MOSQUITO

BY

H A RAGAB, M B CH B, D T M & H, PH D,*
Entomology Department, London School of Hygiene and Tropical Medicine

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During experiments on mosquitoes infected with the malarial parasite *Plasmodium gallinaceum*, I attempted to isolate the gut of the infected mosquito, perfusing it with some nutrient medium and observing the oocysts Some technique was devised to allow for the microscopic observation of the preparation during the perfusion

Very little is known about the characteristics of the body fluids of insects in general and mosquitoes in particular As this body fluid is an important factor which determines the vitality of the various tissues it bathes, it is clear that it contains substances necessary for the living cells Its various salts, their percentage, the hydrogen ion concentration, osmotic pressure, various amino acids, vitamins, hormones, etc , must play an important role in maintaining cells and tissues

MUTTKOWSKI in 1923 worked on the blood of insects and its relationship to respiration and coagulation He found that the reaction of insects' blood was " slightly alkaline or neutral to moist litmus paper " He used various insects but not mosquitoes HABER in 1926 performed similar work on the

* I wish to express my thanks to Professor P A BUXTON for his suggestions and help and to Mr S A SMITH for making the special slide used in the perfusion experiments

common household German cockroach and other insects, but again not mosquitoes.

CROZIER (1924) worked to determine the hydrogen ion concentration within the alimentary tract of some insects. He maintained larvae of *Psychoda* and of *Chironomus* in solutions of appropriate indicators and estimated the pH of different parts of the gut colorimetrically. GLASER (1925), BODINE (1926) and BISHOP (1926) used various techniques in attempting to determine the constitution of insects body fluid. FREW (1928) tried insect tissue culture, using the blow fly larvae. TRAGER (1935a) reported the cultivation of the virus of grasserie in silkworm tissue culture using a synthetic medium he made and kept the virus alive for 2 to 3 weeks. In the same year (1935b) he succeeded in breeding mosquito larvae free of living micro-organisms. Later in 1938, TRAGER cultivated the virus of equine encephalomyelitis in mosquito tissue *in vitro* using larvae and adults of *Aëdes aegypti* brought up according to his previous aseptic technique. In 1941 NAUCK and WEYER successfully maintained rickettsia bodies in louse tissue *in vitro*. They used in their aseptic technique human, guineapig or rabbit plasma at a pH varying from 6.7 to 7.2.

During the progress of my own work, BALL (1947) described a method by which the isolated gut of *Culex tarsalis* previously infected with *Plasmodium relictum* was kept alive for an average period of 7 days. He reported no growth in the oöcysts. These experiments are similar in principle to my own. He used two main types of media: one essentially like that used by TRAGER (1938) and the other a synthetic medium as used by KIDDER and DEWEY (1945). In TRAGER's medium the mosquito midgut showed contractility for 7 days. BALL reported (in personal communication) that except for the fact that the synthetic medium was more constant in composition I found no improvement in it for keeping the oöcysts alive as compared with TRAGER's medium. The pH was 7.2 in the beginning and 6.9 at the end of one of the experiments which lasted 3 weeks.

TECHNIQUE AND METHOD

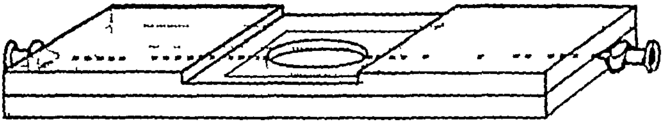
One may mount the dissected gut under a cover slip sealed with vaseline. To carry the perfusing fluid to the preparation, I pulled out a very fine long (30 cm.) capillary tube; the length of the capillary was useful in permitting various movements without breakage. The top of the capillary was put under the cover slip secured to the slide with plasticine and the wide end of the tube attached to a reservoir containing the medium, elevated about 30 to 40 cm. The overflow from the chamber was through another capillary and a piece of filter paper inserted in the chamber.

At first no aseptic precautions were taken but later when bacterial contamination became troublesome various steps were taken to help to overcome it. The reservoir, slides, covers, needles, etc. were autoclaved, the medium boiled or autoclaved (if Tyrode's, it was autoclaved in two parts, then mixed when cool as modified by FLEISCH and quoted by LEE (1937).) The mosquito was handled with sterile forceps, and 70 per cent. alcohol dropped on it for 5 minutes with a sterile pipette. Sometimes it was dipped for 1 minute in a solution of penicillin (100 units per) or in an aqueous solution of iodine (1 : 1000) and subsequently washed by dropping on it from another sterile pipette drops of the sterile medium for about 5 minutes to wash off the antiseptics. Then the mosquito was put on a sterile slide and the gut dissected out and put on another slide under a cover slip

sealed with sterile wax, with inflow and outflow capillaries The continuous flow of sterile nutrient medium was found to help in keeping the preparation sterile or nearly so Penicillin (1 unit per c c) was sometimes added to the medium This seemed to help in eliminating bacterial contamination but shortened the period of life of the preparation and seemed to be toxic Smaller doses seemed unable to stop bacteria and larger doses were definitely toxic

Another technique to overcome bacterial contamination was devised The insect was handled aseptically as before, but instead of dissecting the gut out of it, a window (sometimes all the wall of the abdomen) was cut away to expose the gut without injuring the alimentary tract The exoskeleton was broken away bit by bit with two sterile needles (burnt every now and then) The insect with its exposed gut was then transferred to the perfusion chamber

During the work, the cover slip often got removed from the slide, owing to the capillary tube dislodging it To avoid this a special slide was made of the plastic material Perspex 1



It was the size of an ordinary slide, and was constructed of two sheets of perspex grooved to carry hypodermic needles, for inflow and outflow In the middle of the upper sheet a circle was cut out to serve as observation chamber The middle portion of the upper surface of the top slide was ground away so as to make the chamber shallow An ordinary hypodermic needle (stainless) was put in the channel between the two slides on each side, and the two slides were cemented together Rubber tubing could then be fitted on the needles' heads A regulating clamp was adjusted on the inflow side to allow 4 to 5 drops per minute

This slide was kept in alcohol (70 per cent) for 1 day to sterilize it before use This had no deleterious effect on the plastic material Exposure for long periods tended to soften it

MEDIUM

Different media were tried to find which allows for the longest life of the perfused or transplanted preparation The results are seen in the accompanying table The mosquitoes used were sometimes *Anopheles maculipennis atroparvus* and sometimes *Aëdes aegypti* These latter were sometimes infected with *P gallinaceum* and showed oöcysts (5 to 6 days old)

Oöcysts were measured at the beginning and end of the experiment, when no more gut contractions were visible The measurements showed that the oocysts did not grow

Several times I worked with recently fed mosquitoes, with the midguts distended with blood The result was disappointing as the medium tended to pass into the gut due to differences in osmotic pressure, and finally the gut burst in 10 to 15 hours

Carrel flasks were tried but the water condensed upon the "ceiling" of the flask and thus obscured observation of the preparation Also, due to the depth of the flask, the preparation could not be seen with the high power of the microscope unless taken out of the flask

TABLE.

SHOWING VARIOUS METHODS OF TRANSPLANTATION, MEDIA USED AND RESULTS ARRIVED

Technique	Medium.	pH.	Average hours of longevity	Comment.
I Continuous perfusion either on simple slide or chamber slide (no aseptic precautions)	Saline	7.2	10	Bacteria existed in all these experiments in the beginning and the end. The preparation was nearly eaten up at the end of each experiment, some time after its death.
	Ringer	7.2	24	
		7.4	24	
		6.8	24	
	+ glucose 0.4%	6.8	30	
	Tyrode + glucose	7.2	60–70	
	+	6.6	24	
	+	6.2	8	
	Ball' medium	6.6	10–12	
II Continuous perfusion either in chamber slide or in special slide (aseptic technique)	Tyrode + glucose 0.2%	6.6	48–72	Bacteria were not apparent in the beginning of the experiments but in the end they were seen. In the penicillin experiments, sometimes they were seen and sometimes not.
	Ringer 0.2%	7.2	48–72	
	Tyrode + 0.5%	7	48–72	
	+ 0.8% + penicillin 1 unit per c.c.	7.2	1 25	
	Tyrode glucose 0.8 + penicillin 200 units per c.c.	7.2	24	
	Tyrode glucose 0.2% peptone 1	6.8	4	
	Ball' medium	6.8	48	
	Trager medium	6.8	48–72	
III Hanging drop (aseptic technique)	Tyrode glucose 0.2	6.8	45	Bacteria could be seen at the end of the experiment though not at the beginning, except with 200 units of penicillin per c.c. they did not show at all.
	Ball' medium	6.8	4	
	peni-cillin 1 unit per	6.8	24	
	Trager medium	6	4	
	Tyrode glucose penicillin 1 unit per c.c.	6.8	24	
	Tyrode glucose penicillin 200 units per c.c.	6.8	4	
	Tyrode chick plasma 1:1	7	4–30	

TABLE—*continued*

Technique	Medium	pH	Average hours of longevity	Comment.
IV Special dissection method (*a*) as hanging drop (aseptic technique)	Tyrode " + glucose 0 3% Trager s	6 8 6 8 6 8	72 96 72–96	Bacteria showed sometimes after the experiment ended (*i e*, after the apparent death of the gut as judged by stoppage of contraction) Sometimes the preparation looked the same (not broken up and no bacteria though no contractions for up to 4 to 6 weeks)
(*b*) with continuous perfusion using special slide	Tyrode , + glucose Trager s	6 8 6 8 6 8	72 96 96	Same as above though bacterial growth was less met with

N B—In the above experiments sometimes the insects were infected with *Plasmodium*, they showed oöcysts (5 to 6 days old) at the time of the transplantation The oöcysts did not develop further judging by their size

I also used the ordinary hanging drop method in "sealed" chamber slides, without perfusion as described above It gave quite satisfactory results comparable to the perfusion ones although bacterial contamination tended to occur sooner The best results were obtained when a window had been cut in the insects' abdominal wall

RESULTS

The results arrived at are tabulated showing the technique used, medium, pH, result (as average hours of longevity of the gut, judged by its contractions)

DISCUSSION

The cultivation of the asexual stage of the malaria parasite *in vitro* has succeeded several times As to the cultivation of the sexual stage, nothing has been done until lately BALL (1947) has now described his tests with *P relictum* in *Culex tarsalis*

I was working with *P gallinaceum* in *Aëdes aegypti* and the results,

though of a preliminary nature, are promising. Further work, especially in bio-chemistry is required. In particular we want to know more of the body fluid of the various mosquitoes as this has a direct bearing on the growing parasite.

With regard to the contamination of the preparation with bacteria which eventually leak out of the gut (however aseptic the dissection technique may be), progress might come in the following way the mosquitoes must be bred aseptically kept in a sterile cage, and fed on sterile material. Then, they should be fed on blood drawn aseptically from an infective host, and offered in sterile tubes or through an artificial membrane. In this way it seems to me that even if the gut leaks, or even if it is cut through, there will be no chance of contamination. It is my intention in the near future to carry out some experiments on these lines. Neither BALL nor I could see any development in the transplanted oöcyst.

SUMMARY

Attempts to cultivate the sexual stage of *P gallinaceum* on the stomach of *Aëdes aegypti in vitro* showed

(1) That the stomach could remain alive (contracting) *in vitro* for as long as 4 days

(2) the oöcysts ceased to develop.

REFERENCES.

BALL, G H. (1947). *Amer J trop Med.*, **27** 301
BISHOP G H. (1926). *J biol Chem.*, **69**, 77
BODINE, J H. (1926) *Biol Bull.*, 51 363.
CROZIER, W J (1924). *J Gen. Physiol.*, **6**, 289
FREW J G H. (1928). *J exp. Biol.*, **6**, 1
GLASER, R. W (1925). *Ibid.*, **7** 599
HAGER, V R. (1926). *Ibid.*, **21** 61
KIDDER, G W & DEWEY V C. (1945). *Physiol. Zool.*, **18**, 136.
LEE, A. B. (1937). *The microtomist' vade-mecum.* 10th Ed., London.
MUTTKOWSKI R. A. (1923). *Bull. Brooklyn ent Soc.*, **18**, 127
NAUCK, E. G & WEYER, F (1941). *Zentralblatt f Bakt Parasit. u. Infektion* (Abt. 1), 147 365.
TRAGER, W (1935a). *J exp. Med.*, **61** 501
——— (1935b). *Amer J Hyg.*, **22** 18.
——— (1935). *Amer J trop. Med.*, **15** 337

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CORRESPONDENCE.

THE RATE OF DISAPPEARANCE OF *LEISHMANIA* IN KALA-AZAR PATIENTS UNDER UREA STIBAMINE THERAPY

To the Editor, TRANSACTIONS *of the Royal Society of Tropical Medicine and Hygiene*

SIR,

The above paper* on which the results of treatment of as large a number as 793 patients with kala-azar are reported must necessarily command attention The present writer, therefore, feels that the implications and conclusions of this paper must not be allowed to go unchallenged

The statement " In the past, evaluation of drugs for treating kala-azar was chiefly based upon clinical impression " is very misleading KNOWLES (1920) and SHORTT and SEN (1923) working with this same drug, urea stibamine, and most of the earlier investigators on whose work kala-azar dosage schemes were based, confirmed the cure not only by examination of smears but by culture of blood, or of liver or spleen puncture material The present writer used these parasitological methods to test cure from 1920 until about the end of that decade, but in 1924 he pointed out the limitations of this method , later, when using the short concentrated courses of pentavalent antimony, especially of neostibosan, he found that immediate post-treatment spleen or liver puncture gave no indication at all of the final result, and he abandoned the methods as inflicting unnecessary pain on the patient.

While he does not deny that sternal puncture, a method he himself has used for many years, has a place in the diagnosis of kala-azar, the writer seriously questions the authors' two premises " first, although *Leishmania* are more readily found in spleen puncture, the number of the parasites present in the bone marrow smear, prior to treatment, is more or less in proportion with the degree of infection , second, the parasites in bone marrow seem to be more resistant to treatment "

* HO, EUTROPE A , SOONG, TSUNG-HSIN and LI, YOUNG *Trans R Soc trop Med Hyg* 42, 573

The authors classified their cases according to the number of parasites in the sternal puncture smears and then repeated the puncture after the second, fourth and sixth injection they noted that the fewer the parasites the more rapidly these disappeared (this is not surprising), and also that they disappeared after a smaller number of injections when these were given at weekly intervals instead of twice weekly They therefore concluded that weekly injections were more effective. This conclusion is not only in direct opposition to modern concepts on chemotherapy but also to all of the writer s 25 years experience in the treatment of kala-azar in which he has satisfied himself again and again that concentration of the prescribed antimony dosage within as few days as possible should be exercised to the limit of safety (This principle has since been carried further than he was prepared to take it.) He therefore examined carefully the figures presented by these authors on Table I This table shows that in group 5 sternal punctures after the second, fourth and sixth injections showed absence of parasites in 0 14 7 and 33 3 per cent. of cases respectively when injections were given twice weekly and in 6 7 54 2 and 82·9 per cent. when they were given at weekly intervals. These figures seem to prove their point but the time factor is not taken into account. The present writer has from 1924 onwards repeatedly pointed out that this is very important. The authors did these sternal punctures immediately prior to the next injection so that in the twice-weekly series the punctures were done at the end of 1 week, 2 weeks and 3 weeks (possibly 3½ weeks), and in this case of the weekly injections at the end of 2, 4 and 6 weeks. Now if the percentages of negative sternal puncture smears are re-arranged according to the time interval from the first injection, they are as follows —

Weeks from first injection	1	2	3	4	6
Percentage negative	0	6 7 and 14 7	33 3	54 2	82·9

From this it could well be argued that the time factor was the important one and that, in the one instance in which the time interval was the same the percentage was in favour of twice-weekly injections. This holds for the other groups except those in which there were no or only scanty parasites, and in the latter the two percentages after 2 weeks were about equal.

The authors claim that in their "follow up they found that 1 3 per cent. of cases on weekly injections and 4 1 per cent. of those on twice-weekly injections, had relapsed. In Table IV but not in the text, it is stated that these were cases on which *Leishmania* had disappeared at the conclusion of treatment. The table gives a total of 366 cases on twice-weekly injections. But there were only 344 originally of which 59 had positive sternal punctures at the conclusion of treatment. There is admittedly a footnote to this table noting that additional cases are included in this table and that some did not complete the full course of treatment, but as we know nothing about these additional cases the significance of the percentages 1 3 and 4 1 is lost We are not told what happened to the 59 cases with positive sternal punctures at the conclusion of treatment,

whether they were given further treatment or whether they relapsed clinically In the present writer's experience the relapse rate was very little higher among cases showing a "positive" puncture at the conclusion of treatment than among those showing a negative one

While the writer does not claim that the authors' main conclusion, that weekly injections are more effective than twice weekly, is untrue, he considers that it would be dangerous to accept this on the data presented, and without further confirmation

The authors made three recommendations —

"(*a*) The number of *Leishmania* present in the bone marrow smear prior to treatment be taken as a simple index of the severity or degree of infection"

This recommendation is based on a premise, not on any data presented in this paper It must be assumed that either clinical severity or resistance to treatment is meant In neither instance is this in conformity with the present writer's experience

"(*b*) The rate of their disappearance under therapy be taken as a means to estimate the potency of drugs"

This is possibly sound as a general principle, but there are many exceptions It is in no way supported by the data presented

"(*c*) Their final disappearance be taken as a simple criterion of cure"

Again, this has no relation to the data presented The "final disappearance" could only be ascertained by cultural methods, and even then there will be many instances in which a false conclusion will be arrived at (*e g*, see MORTON and COOKE, 1948)

I am, etc ,
L EVERARD NAPIER

3, Mandeville Place, W 1
5*th July*, 1949

PALUDRINE RESISTANT FALCIPARUM MALARIA

SIR,

The letter from Professor FAIRLEY which appears in your May number discusses the difference in the response to paludrine of two strains of *Plasmodium falciparum*, one from New Guinea and one from Lagos in West Africa The New Guinea strain is extremely sensitive to paludrine, acute infections are—or were 3 years ago—relieved by a single dose of 100 mg and cured by a 10-day course of 3 0 gramme (FAIRLEY, 1946) Yet the trials of COVELL *et al* (1948) in London show that Lagos-strain infections are seldom cured by a

7-day course of 2 1 gramme or a 14-day course of 4 2 gramme New Guinea lies east of Wallace s Line. The difference in strain behaviour suggests FAIRLEY may be related to geographical isolation.

We are tempted to ask whether an acquired difference may come into the picture. Malaya lies on the other side of Wallace s Line. Three years ago falciparum infections observed by us in Malaya were as sensitive to paludrine as New Guinea infections. With almost incredulous surprise we had read FAIRLEY s report of the amazing effects of so minute a dose as 100 mg We repeated his trials with naturally acquired Malayan infections and obtained the same result. All of 28 cases of acute falciparum infection were cured clinic

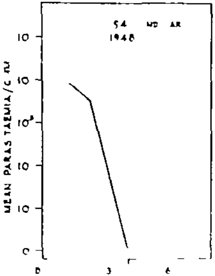

FIG. 1.

Mean daily parasitaemia in 28 cases of acute falciparum malaria receiving single dose of 100 mg

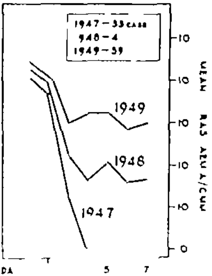

FIG. 2.

Mean daily parasitaemia in 133 cases of acute falciparum malaria receiving single dose of either 250 or 300 mg.

lly by a single tablet of 100 mg and in all cases the parasitaemia cleared within days. There was here we thought, as did FAIRLEY before us, a simple safe powerful means of easing the burden of malaria in scattered rural communities.

That was in 1948 now we are less confident. There were no failures in 1947 and early 1948. The dose was then 100 mg But in the first half of 1949 one out of every four cases treated with from two to three times this dose, failed to respond. The strain was beginning to resist the minute doses hitherto effective

the State of Negri Sembilan The trials were made at the Tampin Malaria Branch of this Institute in the State of Negri Sembilan The patients were drawn from the surrounding countryside mostly from rubber estates which, between 1947 and 1949, had used paludrine for prophylaxis The local strains had been exposed to the drug, regularly or sporadically, for 2 years

The West African strain was brought to England in October, 1947 Did the strain make its first acquaintance with paludrine in London or had there been a significant contact with the drug in Lagos beforehand? Is it possible that an early sensitivity was already beginning to lessen when the strain reached England?

Natural differences in sensitivity to paludrine between the West African and New Guinea strains of *P falciparum* there may be, but, far more important, we suggest, are the acquired differences which may arise in the same strain —important because nothing less than the future use of paludrine as a falciparum schizonticide is at stake Drug resistance is a stage in a dynamic process None can say how far it will go, whether, indeed, it will progress until the least dose which destroys the parasite is greater than the host can tolerate ADAMS and SEATON (1949) have produced a strain of *P falciparum* by serial blood inoculation and constant exposure to paludrine, which at the tenth passage resisted a 10-day course of 1 0 gramme daily This is already near the limit of safe dosage for the human host We ourselves have observed a change under less artificial conditions from the high sensitivity of 2 years ago, when 100 mg was enough to clear the blood of asexual parasites, to a resistance which was so marked in one case recently observed that more than one hundred times this dosage failed to clear the blood for more than a few days (Fig 3)

Where will this rising resistance end? Will it progress until paludrine

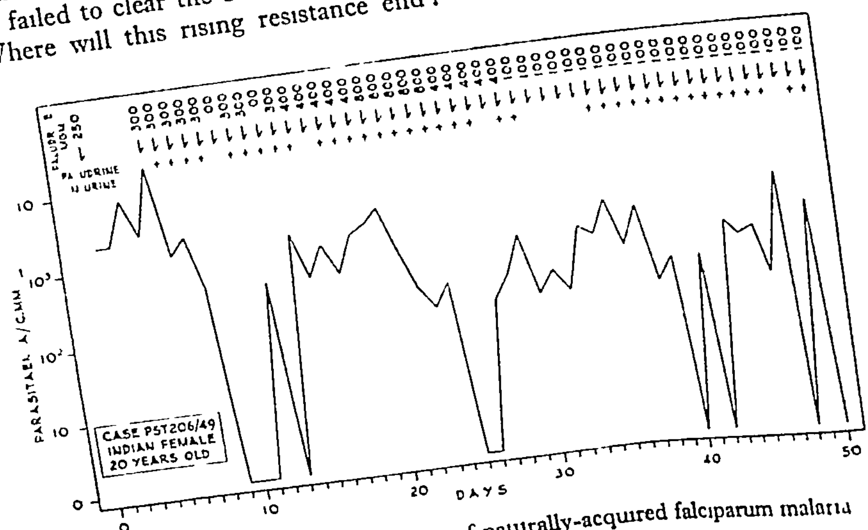

FIG 3 —Paludrine-resistance in a case of naturally-acquired falciparum malaria

ceases to be a useful schizonticide in falciparum malaria? This is a problem we have to face in Malaya. The medical services, the Army and the rubber estates have used the drug with confidence and success, though less, perhaps for therapy than for suppression. That success, on the whole is maintained. We ourselves have kept an estate population on suppressive paludrine for 2 years with a fall in the overt malaria of 80 per cent. compared with controls. Post-suppressive infections treated at ordinary therapeutic dosage at the end of this time were still sensitive to the drug. Whatever changes in sensitivity there may have been, here and throughout Malaya as a whole, are still, as it were hidden below the surface of current dosage. For how long will they stay there? They have broken the surface in the Tampin district they may do so elsewhere and a prophylactic weapon hitherto so sharp will then turn blunt in our hands.

We are, etc.,
J W FIELD.
J F B. EDESON

Institute for Medical Research,
Kuala Lumpur
23rd July 1949

REFERENCES.

ADAMS, A. R. D & SEATON D R. (1949). *Ibid.*, 42, 315
COVELL, C., NICOL, W D SHUTE P G & MARYON, M. (1949). *Ibid.*, 42, 465
FAIRLEY N H. (1946). *Trans. R. Soc. trop Med. Hyg* 40 105
——— (1949). *Ibid.* 42, 623.

FILARIA IN THE SUDAN

SIR,—

I was much interested in Dr WOODMAN's paper on Filaria in the Sudan (*Trans R. Soc trop Med. Hyg* 42, 543). My own observations do not altogether coincide with his, and I feel that the following points should be recorded.

(*a*) The vector of *Loa loa* My own experience is that *Chrysops* spp did attack man and could be found commonly near habitations. They were a common nuisance in the Li Rangu leprosarium, the most thickly populated part of Zande area of which he writes. During tsetse fly surveys carried out on foot they would also attack carriers. The Zande have a special name for these flies, which suggests that they are common attackers of man.

(*b*) The fact that Europeans have been infected with loiasis without

being aware of having been bitten by *Chrysops* is of little importance Unless the person concerned was able to identify the fly such data are of no value It only serves to stress that even the most vicious insect biter can bite without viciousness on occasion

(*c*) The distribution of onchocerciasis he quotes is not correct A large area, approximately 10,000 square miles in area, and lying south of the words "Raffile Rapids," and between the Sueh and Naam rivers, is uninhabited, and onchocerciasis is not endemic there This area was chosen as a game reserve because it was uninhabited, and one of the features of the area is a lack of water except at a few places Neither onchocerciasis nor other filariae are likely to be found there, nor is man

(*d*) Onchocerciasis also exists along the Ethiopian border from the map reference "Pibor" northwards to opposite the map reference "Melut" I myself have found cases (a 30 per cent infection rate taking skin scrapings), and the fly, *Simulium damnosum*, which was identified by the medical entomologist, Mr D J LEWIS

(*e*) In selecting cases for examination for onchocerciasis, Dr WOODMAN includes some "eye cases" None of these had any clinical examination to prove this diagnosis, and it is impossible to make such a diagnosis without such an examination For ocular onchocerciasis, RIDLEY (1945) gives the most comprehensive description of diagnostic methods and clinical signs (There is, incidentally, no reference to this paper) I myself have seen cases of choroidal retinitis due to onchocerciasis, diagnosed with an ophthalmoscope, in the Tembura area

(*f*) Dr WOODMAN suggests that the heavy infection causes the ocular lesions RIDLEY (1945) notes that the ocular lesions are commonest where the nodules are near the eye, and that they usually appear late in the disease They have been seen in children aged 4, though probably most eye lesions ke 5 years to develop WOODMAN does not mention anything about the site the nodules in his "eye cases"

(*g*) From my own experience, I think that the intensity of infection has something to do with the incidence of the eye lesions In the Sudan the disease is commonest in the Raga, Raffile, Rumbek and Mvolo areas Here the people live along rivers, which form ideal breeding grounds for the fly, and the fly can be found all the year round They take their drinking water from these rivers, wash in them, and fish in them continually In the Zande area to the south, where the disease is thought to be less common, the conditions are different The people use continually the smaller tributaries, where the fly does not breed regularly They go to the larger rivers such as the Sueh in certain seasons only Their chances of infection are therefore only seasonal The site marked Sueh 11 in Dr WOODMAN's map is the one exception to this rule and has for many years been known as a highly endemic area

(*h*) Onchocerciasis as a cause of large hydroceles has been proved in the Belgian Congo. *Loa loa* is a connective-tissue parasite and it would not be unusual to find it in the large hydroceles, but that would not prove that it caused the hydroceles.

(*k*) The table on page 548 is a little misleading The number of persons examined should be 148 and not 1440 The percentage of blindness quoted is of no significance as the eye cases were not proved to be due to onchocerciasis.

The incidence of onchocerciasis in the Sudan in the area he covers is serious and this has been known for over 20 years. Accurate evidence of its incidence has only recently been forthcoming and this is due to the work of Dr R. KIRK, Assistant Director of Research, and Mr D J LEWIS, medical entomologist, to the Sudan Government. They have done a full survey in the area and it is to be hoped their findings will be published, for there is need for some more accurate information on this subject.

I am, etc.
J F E. BLOSS.
Province Medical Inspector Sudan Medical Service

Malakal.
26th July 1949

REFERENCE.

RIDLEY H (1945). Ocular Onchocerciasis. *B J Ophthalmology* Monograph Supplement X.

Printed in Great Britain by H. K. [illegible] Ltd. [illegible]

ANNOUNCEMENTS.

NEXT MEETING OF THE SOCIETY

The next meeting, the Opening Meeting of the 43rd Session, will be held at Manson House on Thursday, 20th October, 1949, at 7 30 p m Professor H E SHORTT, C I E, M D, will deliver his Presidential Address, entitled "Tropical Medicine as a Career"

MANSON LECTURE

To perpetuate the memory of the late Sir PATRICK MANSON, the Council of the Society has decided to establish a MANSON LECTURE FUND, to which subscriptions are now invited It is hoped to raise a sum of at least £2,500, the accumulated interest from which will be devoted to financing a Manson Lecture

The Lecture will deal with some aspect of tropical medicine or hygiene and will be given periodically by a recognized authority The lecturer and the subject on which he will be invited to speak, will be decided by the Council of the Society

The Manson Lecture will be open to all who are professionally interested and will be advertised in the general medical press, in which it may be subsequently published

MOVEMENTS OF FELLOWS

The following Fellows from abroad have notified the Secretaries that they are *temporarily* in the British Isles Letters addressed to any of these care of the Royal Society of Tropical Medicine and Hygiene, Manson House, 26, Portland Place, London, W 1, can usually be forwarded to the home address

To ensure the accuracy of this list, Fellows named below are particularly requested to advise the Secretaries when they return to their stations abroad

ABBOT, P H, Sudan
ABDEL MESSIH, Egypt
AGRAWAL, J P, India
AJOSE, O A, Nigeria
ANDERSON, N, New Guinea
APTED, F I, Sierra Leone
AWOLIYI, S O, Nigeria
BANNERMAN, E W, Gold Coast
BARNES, G T, Fiji
BLOMFIELD, D M, Kenya
BRAINE, G I H, Malaya
BURKE, M E T, Assam
CALWELL, H G, Tanganyika
CAMPBELL, G, Trinidad
CHAO, WEI-HSIEN, China
CHILTON, N, Tanganyika
COPELAND, F J, India
COOPER, P R, Nigeria
COSGROVE, P C, Sierra Leone
DAVIDSON, Lt -Col T J, India
DICKIE, ROBERT, Nigeria
DOMAINGUE, F G, Mauritius
FARMAN-FARMAIAN, S, Persia
GOH, K A, Hongkong
GELFAND, M, S Rhodesia
HADDEN, W E, Gambia
HARDING, R D, Nigeria
HAWE, A J, Gold Coast
HILL, K R, U S A
HOLMES, R E, Belgian Congo
HOWARD, A C, Cyprus
HUNTER, W, Nigeria
INNES, J ROSS, Tanganyika
KELSEY, H A, Nigeria
LESH, J I, Nigeria
LE SUEUR, E, Sarawak
LOW, NAN-WAN, Malaya
LWIN, R, Burma
MACGREGOR, R B, Malaya
MACNAMARA, F N, Nigeria
MADGWICK, G A S, South Africa
MOK, HING YIU, Hongkong
MWAISELA, E F, Tanganyika
NICHOLLS, L, Singapore
PASQUAL, J R H, Nigeria
RAM, J W, Burma
RAPER, A B, Uganda
REED, J G, Malaya

Movements of Fellows—*Continued*

RENNER, E. A., Sierra Leone.
RITCHIE, G. L., Tanganyika.
RUSSELL, A. F., China.
SEAL, K. S., Nigeria.
SIRKAR, S. C., India.
SHAH, FULCHAND, India.
SHEARER, G. Nigeria.
SIMPSON, T., Nigeria.
SIU KA-HEE, Hongkong.
SUR, M. L., India.
TO SHIU-YUEN, Hongkong.
TWINING, H., Borneo.
URS, BASAVARAJ, India.
VAN-DE LINDE, P. A. M. Hongkong.
WILSON CARMICHAEL, Nigeria.
WILSON, T. Malaya.
WOODMAN, H., Sudan.
WORTH, H. N., South Africa.
YAO K. C., Hongkong.

NEW FELLOWS

At the meeting of the Society held at Manson House on 1st July 1949 the following 28 candidates were elected Fellows of the Society :—

ANDREASEN A. T. F.R.S.E., F.R.C.S., England.
BAIRD, STEPHEN Y. M.B., CH.B. (GLAS.), Assam.
BENNETT JAMES P., M.B., B.S. (DURHAM), D.OBST.R.C.O.G., Sarawak.
CASTILLO, ROBERTO L., B.SC. BIO., M.P.H. M.D. PH.D. Ecuador.
CHEWE, WILLIAM, M.SC. (LIV.), British Cameroons.
DAVIS, T. R. A., M.B., CH.B. (N.Z.), Cook Islands.
FRATER, A. S. M.B.E., M.B., B.S. (MELB.), D.T.M. (SYD.), Fiji.
GARCIA, ONOFRE, M.D. (SANTO THOMAS), Philippines.
HALL, CHRISTOPHER L., B.A., B.M., CH. (OXON.) M.R.C.S. (ENG.), L.R.C.P. (LOND.), Tanganyika.
HALSTEAD BRUCE W. M.D. (CALIF.), U.S.A.
HAWORTH, JAMES M.B., CH.B. (EDIN.) Nigeria.
HOLSTEIN MAX H., B.SC., Medical Entomologist, French West Africa.
JONES, GERALD E. S., B.M. B.CH. (OXON.), Sierra Leone.
KOTHARE, K. G. (Miss) D.A.B.F. (BOMBAY), L.M. (DUBLIN), India.
LOH, MUNG SUN, L.R.C.P. & S. (GLAS.), L.R.C.S., L.R.C.P. (EDIN.) Sarawak.
LAPEYSSONNIE, LEON M.D., A.D.F.
MCKINLAY D. M.R.C.S. (ENG.), L.R.C.P. (LOND.) Sierra Leone.
MAHINTURA, G. J. L.R.C.P. & S. (EDIN.), L.F.P. & S. (GLAS.), India.
MOKAR, H. N. M.D. (CALIF.), U.S.A.
NIMALASURIA, A. M.B., B.S. (LOND.), M.R.C.P. (LOND.), Ceylon.
ONG, KIM TEN L.M.S. (SINGAPORE) Sarawak.
PILLAI P. P. G. M.B. B.S. (MADRAS), Sarawak.
ROBERTS, FRANK R., L.R.C.P. L.R.C.S. (EDIN.), L.R.F.P. & S. (GLAS.), T.M. & H. (ENG.), Gold Coast.
ROBINSON MARION C. M.A. (OXON.), B.M., B.CH. (OXON.), D.T.M. & H. (ENG.), England.
SABRY IBRAHIM, Prof. Dermatology and Venereal Disease, Alexandria.
SANDOSHAM, ARTHUR A., L.M.S. (SINGAPORE) Sarawak.
SANGITOAM, C. L.M.S. (SINGAPORE), Sarawak.
WALLACE, EDWIN H., M.B., CH.B. (GLAS.), Sarawak.

LIBRARY NOTICE

Owing to lack of accommodation in the Library it has been found necessary to dispose of duplicates and old editions. The following books are offered free to Fellows either on application in person or forwarded on payment of postage.

ALLBUTT T. CLIFFORD A System of Medicine Vol. II (1897).
ALLBUTT T. CLIFFORD, & ROLLESTON, H. D. A System of Medicine Vol. I (1906) and Vol. II (1907).

Library Notice—*Continued*

ALPORT, A C Nephritis (1929)
ANDERSON, D E Epidemics of Mauritius (1918)
ANIGSTEIN, LUDWIG Tropical Typhus (1933)
ASHFORD, B K Soldier in Science (1934)
AVERY, HAROLD Gastric and Duodenal Ulcers (1940)
BABSKY, E B, KOCHERGIN, I G, & PARIN, V Microbiology and Epidemiology (1945)
BAGSHAW, WALTER Elementary Photo-Micrography 2nd ed (1909)
BECKMAN, HARRY Treatment in General Practice (1931)
BERTRAND, L E, & FONTAN, J De l'entero-colite (1887)
BESREDKA, A Immunity in Infection Diseases (translated from the French, by Herbert Child) (1930)
BEESON, A Practical Bacteriology, Microbiology and Serum Therapy (translated from the French by H J Hutchens) (1913)
BLACKLOCK, D B, & SOUTHWELL, T A Guide to Human Parasitology 1st and 2nd editions
BLACKLOCK, MARY G An Elementary Course in Tropical Hygiene Parts I and II (1926)
BLUMFELD, J Anaesthetics (1902)
BOSE, C L The Scientific and Other Papers Vol I (1924)
BRABANT, A B Colonization of Indo-China (translated from the French by J Chailley-Bert) (1894)
BRAUN, M, & LUHE, M A Handbook of Practical Parasitology (1910)
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BROWN, W LANGDON Physiological Principles in Treatment (1924)
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CALMETTE, A L'Infection Bacillaire et la Tuberculose (1920)
CAMMIDGE, P J The Faeces of Children and Adults (1914)
CANNON, ALEXANDER, & HAYES, E D The Principles and Practice of Psychiatry (1932)
CASTELLANI, A & CHALMERS, A J Manual of Tropical Medicine 1st ed (1910) and two 2nd editions (1913)
CHANDLER, A C Introduction to Human Parasitology 5th ed (1936)
CHESNEY, A M Immunity in Syphilis (1927)
CHESTERMAN, C C African Dispensary Handbook 3rd ed (1937)
CHESTERMAN, C C In the Service of Suffering (1940)
CHITTENDEN, R H Physiological Economy in Nutrition (1905)
CLAY, HENRY H Sanitary Inspector's Handbook 3rd ed (1937)
COBBETT, LOUIS The Causes of Tuberculosis (1917)
COCA, ARTHUR F Essentials of Immunology (1925)
COLES, ALFRED C The Diseases of the Blood (1905)
COLYER, J F Dental Disease in its Relation to General Medicine (1911)
COVELL, GORDON Malaria Control by Anti-Mosquito Measures (1931)
CRAIG, CHARLES F Parasitic Amoebae of Man (1911)
DALE, HENRY Some Epochs in Medical Research (Harveian Oration) (1935)
DANIELS, C W Laboratory Studies in Tropical Medicine 1st ed (1903)
DANIELS, C W, & STANTON, A T Laboratory Studies in Tropical Medicine 2nd ed (1907)
DANIELS C W, & NEWHAM, H B Laboratory Studies in Tropical Medicine 3rd ed (1911)
DANIELS, C W Tropical Medicine and Hygiene Part I (1913)
DEADERICK, W H A Practical Study of Malaria (1909)
DIBLE, J H Recent Advances in Bacteriology (1929)
FAGGE, C H Principles and Practice of Medicine Vol I (parts 1–4), Vol II (parts 5–8) (1888)
FANTHAM, H B & PORTER, ANNIE Some Minute Animal Parasites (1914)
FELDMAN, W M General and Child Haematology (1933)
FINDLAY, G M Recent Advances in Chemotherapy 1st ed (1930)
FLUGGE, C Micro-Organisms (1890)

Library Notices—*Continued*

Flügge, C. Die Mikroorganismen. Vols. I and II (1896).
Gedoelst, L. Synopsis de Parasitologie (1911).
Giles, G. M. Climate and Health in Hot Countries (1904).
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Green, C. R. M. & Green-Armytage, V. B. Birch's Management and Medical Treatment of Children in India. 5th ed. (1913).
Harford, C. F. Mind as Force (1924).
Harford, C. F. Climat. Vols. I, II, III, IV (1902–3) and Vol. V (1904).
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King, D. Barty. Studies in Influenza (1922).
Kirk, J. Balfour. Hints on Equipment and Health (1926).
Langeron, M. Précis de Microscopie (1913).
Le Dantec, A. Précis de Pathologie Exotique. 2nd ed. (1905).
Lehmann, K. B. & Neumann, R. Bakteriologie. Atlas und Grundriss. Teil II (1896).
Leitch, J. Neil. The Use of Standard Treatment in the Campaign against Disease in the Tropics (1929)
Low, G. Carmichael. The Literature of Charadriiformes (1924).
Lukis, P. & Blackham, R. J. Tropical Hygiene for Residents in Hot Climates. 3rd ed. (1915)
Lumb, Norman. Gonococcal Infections in the Male (1920).
McCay, D. The Protein Element in Nutrition (1912).
McDill, John R. Tropical Surgery and Diseases of the Far East (1918).
Mahorner, H. R., Brown, G. E. & Edgar, V. A. Thromboangiitis Obliterans (1928)
Manson's Tropical Diseases. Duplicate copies of the 8th and 10th editions.
Markley, Klare S. Fatty Acids (1947)
Marquez, Luis Cuervo. Geografia Medica y Patologia de Colombia (1915)
Mathews. Physiological chemistry
Mathis, C. & Leger, M. Recherches de Parasitologie et Pathologie (1911).
Mellanby, Edward. The State and Medical Research (1939).
Mitchell, J. P. Anatomy and Physiology and the Causes of Disease (1937).
Miyajima Mikinosuke. Teacher and Pupil (1935).
Monrad-Krohn, G. H. The Clinical Examination of the Nervous System. 2nd ed. (1923).
Moore, N. & Paget, S. Royal Medical Chirurgical Society of London, 1805–1905 (1905).
Morland, Andrew. Pulmonary Tuberculosis in General Practice (1933).
Muirhead, W. Alex. Practical Tropical Sanitation (1914).
Mukhopadhyaya, G. Tropical Abscess of the Liver (1913).
Mummery, P. Lockhart. The Sigmoidoscope (1907).
Musgrave, W. E. & Clegg, M. T. Amebas. Part I. Their Cultivation and Etiologic Significance
Musgrave, W. E. & Clegg, M. T. Amebas. Part II. Treatment of Intestinal Amebiasis in the Tropics (1904)
Nadkarni, K. M. Indian Materia Medica (1927)
Nattan-Larrier, L. Travaux Scientifiques (1913)
Neligan, A. R. The Opium Question (1927).

Library Notice—*Continued*

Nevu-Lemaire, M Humaine Parasitologie (1908)
Newman, George Bacteriology and the Public Health 3rd ed (1904)
Newman, Ehlers & Impey Prize Essays on Leprosy (1895)
Noc, F Technique de Microbiologie Tropicale (1905)
Paget, Stephen Experiments on Animals (1906)
Parkinson, J Rheumatic Fever and Heart Disease (1945)
Parsons, Allan C Encephalitis Lethargica, Report on (1922)
Pembrey, M S & Ritchie, J General Pathology (1913)
Peter, F M & Schmidt, H Ergebnisse und Fortschritte der Antimonietherapie (1937), English translation (1938)
Price, Frederick Textbook of the Practice of Medicine 2nd ed (1926), 4th ed (1933), 5th ed (1937)
Prout, W T Lessons on Elementary Hygiene and Sanitation (1913)
Roberts, Stewart R Pellagra (1912)
Roger, G H, Widal, F & Teissier, P J Avitaminoses, Agents Physiques, Nutrition, (1921)
Roux, Fernand Traité Pratique des Maladies des pays Chauds Parts I, II and III
Ruiz-Arnau, R Lymphectasie Tropicale Primative (1916)
Sabouraud, R Maladies du Cuir Chevelu Vol I (1902), Vol II (1904)
Savage, George H Insanity (1901)
Scheube, V Die Krankheiten der Warmen Länder (1896)
Scheube, V Die Krankheitender der Warmen Länder Translation by P Falcke, edited by James Cantlie (1903)
Sergent, E, Donatien, A, Parrot, L & Lestoquard, F Etudes sue les Piroplasmoses Bovines (1945) Three duplicate copies
Shelley, Horace M An Epitome of the Laboratory Diagnosis and Treatment of Tropical Diseases (1936)
Sherman Chemistry of Food and Nutrition (1937)
Short, A Rendle Index of Prognosis and End Results of Treatment by Various Writers 3rd ed (1922)
Simpson, Andrew Tropical Climates (1820)
Soper, F L, Wilson, D Bruce, Lima, S & Sa Antunes, W The Organization of Permanent Nation-wide Anti-*Aedes aegypti* Measures in Brazil (1943)
Stitt, E R The Diagnostics and Treatment of Tropical Diseases (1914)
Strong, R P, *et al* Onchocerciasis (1934)
Sullivan, John Endemic Diseases of Tropical Climates (1877)
Swellengrebel, N H, & De Buck, A Malaria in the Netherlands (1938)
Thayer, William S Malarial Fevers (1900)
Thin, George Leprosy (1891)
Thompson, C J S Pharmacopaeias and Formularies 7th ed (1933)
Thomson David, & Thomson, R Historical Survey of Researches on the Streptococci (1927)
Thresh, J C, & Beale, J E A A Simple Method of Water Analysis 10th ed (1931)
Waddell, L A Lyon s Medical Jurisprudence for India 5th ed (1914)
Ward, Espine Favourite Prescriptions (1926)
White C F, & Willoughby, H Aids to Sanitary Science and Law 2nd ed (1934)
White, W Hale Materia Medica 14th ed (1915)
Wilson, B McNair British Medicine (1941)

Opuscula Selecta Neerlandicorum de Arte Medica Vol X (1931), Vol XVI (1937)
Selected Essays and Monographs New Sydenham Society (1897)
Health Instruction for Missionaries in the Tropics (1928)
Toxicology of Beryllium Bull No 181 U S Public Health Service (1943)
Report of the Pellagra Commission, 1911 (State of Illinois)
War Areas Memoranda on Medical Diseases in the Tropical and Sub-Tropical Edited by Andrew Balfour (1919)
Ditto (Not war areas) (1930) Edited by W P MacArthur

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YEAR BOOK, 1949

List of Fellows (with Addresses) Alphabetically and Geographically arranged The Society's Annual Reports, Laws, and other matter

Price Five Shillings, post free

MONOGRAPH

Monograph I (September, 1936) "Boomerang Leg and Yaws in Australian Aborigines," by C J HACKETT 66 pages, 17 pages plates, stiff board cover, linen back Price 5s, post free

"MANSON CENTENARY"

Proceedings of meeting at Manson House on 12th December, 1944, with coloured portrait, price Two Shillings and Sixpence, post free

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An illustrated pamphlet by "Onlooker," describing the work and functions of the Society and the amenities of Manson House

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Orders for the TRANSACTIONS and other publications of the Society should be sent direct to the Secretary, Royal Society of Tropical Medicine, Manson House, 26, Portland Place, London, W I *Telegrams* Anopheles, London *Telephone* Langham 2127, Cheques, Postal Orders, etc, should be made payable to the Royal Society of Tropical Medicine and Hygiene

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No 3	November 25th	No 6	May 25th

Advertisement type area 5 inches wide, 7½ inches deep (approx)

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Papers submitted for publication in these TRANSACTIONS should be sent to the Hon Secretaries, Manson House 26, Portland Place London W.1.

The submission of matter for publication will be understood to imply that it is offered to this journal alone.

If accepted for publication, the copyright of papers becomes the property of the Society; but they may be re-published by permission of the Council, provided due acknowledgment be made of their having appeared in the TRANSACTIONS.

Papers should, if possible, be typewritten they should be concisely written with subject matter logically arranged and sub-divided with references and abbreviations in the form described below; and with indications of the position, in the text, of illustrations, tables, maps, etc.

Titles should be as brief as consistent with clarity and in many cases the value of paper is enhanced by short **summary** t the end.

Temperature charts graphs and drawings should be if possible in Indian ink on Bristol board with detail and essential lettering large enough to be clearly legible after reduction if necessary (Write in pencil if lettering on drawing is to be set up and printed.)

Illustrations—if the number sent in is considered excessive, the author may be informed and given the opportunity of contributing to the cost.

Coloured plates are made only at the author' expense.

REFERENCES.

In the text, the date of publication, in brackets, should follow the name of the author quoted thus :—

"To MANSON (1879) is due this epoch-making discovery

At the end of the paper list of **References** should be arranged in alphabetical order of authors surnames, and details given in the following order :—(1) Surname of author; (2) Initials of author; (3) Year of publication, in brackets; (4) Title of article, avoiding arbitrary capitals. (The title of the article is sometimes omitted; but each list of references should in this respect be consistent throughout—giving all titles, or omitting all). (5) Title of journal; (6) Volume number; (7) Page number .g :—MANSON, P (1879). On the development of *Filaria sanguinis hominis* and on the mosquito considered as nurse. *J Linn. Soc. (Zool)*, 14 304.

In the case of reference book (1), (2) and (3) as above; (4) Title of book; (5) Edition and/or volume if more than one (6) Page number; (7) Town of publication; (8) Publisher's name g :—MANSON, P (1895). *Tropical Diseases* 1st Ed 447 London Cassell & Co, Ltd.

Reference to an **Annual Report** SWAZILAND (1937). *Annual Medical & Sanitary Report 1936* p. 10

Note The year of publication is not usually the year covered by the Report.

ABBREVIATIONS.

The abbreviations used are those shown in the **World List of Scientific Periodicals, 1934** which conforms to the rules of the *International Code of Abbreviations for Titles of Periodicals*, Paris, 1930. I general nouns hav capital, adjectives small, initial letters; articles, conjunctions and prepositions are omitted the place of imprint is added only when uncertainty might arise, g

Amer J Hyg	*C R. Acad Sci., Paris.*	*J Pharmacol.*
Ann trop. Med Parasit	*C R. Acad Sci., Johannesburg*	*Ned. Tijdschr Geneesk.*
Arch. Schiff u. Tropenhyg	*Dtsch. med. Wschr*	*Trans. R. Soc trop. Med. Hyg*
Bull. Soc Path. exot	*Indian med. Gaz.*	*Z. Hyg InfektK*

The following contractions are in use whether the number to be expressed is 1 or more, e.g 1 c.c., 15μ. 43 kg) —

centigramme cg	kilometre km.	millimetre, mm.
centimetre, cm	micron, μ	ounce, oz.
cubic centimetre c.c	microgramme μmg.	pound lb.
cubic millimetre, .mm.	milligramme mg	
kilogramme kg	millilitre, ml	

In order to avoid dangerous error in dosage gram and gramme are printed in full.

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NOTICE.—The attention of those who propose to contribute papers for publication is directed to "Editorial Notices" on page xviii.

TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

VOL. 43 — No. 3

NOVEMBER, 1949

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TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

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OPENING MEETING OF THE FORTY-THIRD SESSION
of the Society held at
Manson House, 26, Portland Place, London, W 1,
on
Thursday, 20th October, 1949, at 7 30 p m

THE PRESIDENT,
Professor H E SHORTT, C I E, M D, D SC, D T M & H, Colonel I M S (retd),
in the Chair

PRESIDENTIAL ADDRESS.

In considering what might be a suitable subject for my Presidential Address, I felt a little timorous when I recalled the addresses delivered by my predecessors in this Chair, men to whom I have looked up with reverence as my teachers, men I am proud to succeed as a humble follower

One thing, however, gave me heart of grace I remembered that there is no discussion on the Presidential Address, and one is therefore in the strong position of the broadcaster who can make you furious but to whom, at the moment, you cannot reply! Having thus regained courage, I decided that courage was a good thing and that I must advocate its cultivation

Now, the older generation among us has been through two world wars, and we feel that the world is in a sorry mess I have decided, therefore, to address my remarks chiefly to the younger generation, to those of you who are starting your careers in the midst of this mess It is for you, and it should be your privilege as well as your burden, to take your courage in both hands

and proceed to clear up the mess and it will need courage and resource, but the recompense will be great. The rewards will probably not be wealth or advancement although these may follow but there will be the satisfaction of achievement for its own sake and for the benefits it will shower on others.

Now you may ask what all this has to do with tropical medicine That is a fair question, and my address tonight is largely my attempt to answer it. As my text I take the title of my address Tropical Medicine as a Career From what I have already said you will understand why I address myself tonight chiefly to the younger man starting his career and why I urge any of you in that position who are attracted by the study of tropical medicine seriously to consider making it your life study Such a decision will demand much of you you will have to devote all your talents, all your energy and all your enthusiasm to the task nothing less will do and you can do nothing more. Your choice of career is wide open. Are you interested in clinical medicine? There are vast unexplored fields to traverse. Are you interested in parasitology or entomology? Although the days when you could describe a new species of parasite from every animal caught in nature are past, the amount still to discover knows no end. Are you interested in sanitation? There is a veritable jungle of dirt, disease and destitution to be cleared Are you interested in nutrition and nutritional deficiencies? You have here one of the widest spaces of all to explore.

On the other hand, have you some restless fire in your blood which irks you under the restrictions and conventions of modern civilized life in great centres of population like most European countries? Then think of work in the tropics where open air is cheap and in plentiful supply where you will often be far enough away from headquarters to feel your own master to take responsibility for your own actions, to make your own mistakes and to rectify them because you have no one else to lay the blame upon. This makes for humbleness while you are building up self reliance.

I have said enough, I think, to make it clear that in deciding t take up the study of tropical medicine you are not entering a one-way street leading only at the end of life to a tanned wizened skin, an enlarged liver and retirement to a cottage on a small pension. You are entering a labyrinth of highways and byways each opening out interesting and even enchanting vistas of achievement, and the treading of these roads will leave you with a store of interesting and exciting memories for later digestion in the cottage I have mentioned.

Now I come to a very important aspect of our subject. What are the mental and physical disciplines we must undergo to attain our object of a successful career in tropical medicine? They are the same, whatever may be the line of work chosen among those I have enumerated. Tropical medicine is of course, an extension of general medicine into new territory and, as such,

is almost a new subject. Being practised in new territory much is unknown, and it is, therefore, essential rather than incidental that any approach to it should be made in a scientific spirit of enquiry, whether the student is a clinician, a laboratory worker or a sanitarian. What, then, is this state of mind in which we should approach the problems of tropical medicine? It is, of course, not peculiar to these problems but should become a habit in any scientific enquiry and is called the "scientific method." Let me take, one by one, some of the components which go to make up the scientific approach to any problem. As I enumerate and explain these, you will notice that some of them are closely related, one to another, and that when taken together as a directive they amount to instructions to observe accurately, to criticize sternly and to allow no personal bias to affect logical conclusions.

In the first place, one must be open-minded, casting aside bias and prejudice of every kind. A problem should be approached objectively. There should be an eagerness to consider and weigh all evidence even when it appears to traverse opinions thought to be already established. Failure to do so puts one at a disadvantage compared with one receptive to new ideas and prepared to investigate before discarding them. Thousands of times bacteriologists have found moulds growing on their cultures and thrown them away as contaminated. FLEMING went farther; he looked at his mould and found it was producing something which destroyed the bacteria around it. It appeared an insignificant effect, but it was the germ of a new idea which was not just cast aside. He was open-minded and prepared to accept a possible significance in the phenomenon and the result was penicillin and a whole range of useful antibiotics.

Then one should cultivate what I may call an allergy to problems. This characteristic really differs little from the open-mindedness I have just described. That is to say, one should be quick to see a phenomenon and then to wonder why it occurs. From this naturally follows an attempt to investigate the underlying causes. Millions of people had seen apples fall from trees but they were not allergic to what, after all, was a very remarkable occurrence, viz., the transference of a solid object across space without apparent force applied. Of all the millions who had seen this take place, only NEWTON was allergic enough to sense a problem and to carry the matter farther.

The third essential to the scientific method is a passion for facts as opposed to mere impressions. This is another way of defining the search for accuracy; accuracy in observation and in recording these observations. This striving for accuracy is exemplified by the use of many scientific instruments meant to extend and make more accurate the information given by our senses. A simple example is an instrument such as a ruler, which enables us to say that an object is not merely as long as one's finger but that it is a given number of inches long. Anyone with a ruler can then make comparable observations, and so the

statement becomes strictly accurate and verifiable by others. In the same way the microscope extends and makes more accurate the vision of the eye and the stethoscope the accuracy and range of the ear

The fourth essential to cultivate is a passionless logic and honesty which compels one to give an equal value to all facts, both those proving and those against a favourite hypothesis. It must admit the necessity to retrace steps which have been found to lead along the wrong road and this admission of wrong should be welcomed as keeping one on the right track and in the right faith.

The fifth essential is what I would call controlled enthusiasm which should make your outlook not static but dynamic. This habit of mind will cause us to reach out intellectually and ardently in all directions but also to weigh carefully all the information gained in our search for causes. It will enable us to sift the likely from the unlikely and so ever edge us towards the truth until that is finally attained.

The sixth habit to cultivate is the critical mind. This enables us to consider our findings, both negative and positive, from all points of view All unproved hypotheses, even those generally accepted, must be considered at least suspect until finally proved or disproved, and this applies with special force to one's own hypotheses because we know that we are biased in their favour

The seventh attitude to cultivate is to delay your pronouncements on any problem until you have sufficient proof to make no other conclusion possible. A supreme example of this principle was the collection of data by DARWIN for over 20 years before he enunciated his theory of organic evolution.

The last of your disciplines I would recommend is to work doggedly at your problem along the lines you believe to be correct and not to be discouraged or turned aside when everything seems against you. Try to make your philosophy such that you acknowledge no such thing as failure or defeat that these are only obstacles to make new endeavours necessary Time and again I have found that when things look their worst, when you are discouraged and no gleam appears in the darkness, this is the time to feel hopeful and to look for some relieving light of success. It is often when you are most exhausted and your cause seems hopeless that you exact the greatest forfeit from Nature, who is loath to give up her secrets but, when she relents, rewards abundantly All your work which has gone before is like the sun and the rain on corn. You have without knowing it, been approaching the time of harvest and, one day you find the corn ripe for the garnering

To illustrate this, let me give you a personal experience. I had worked intensively at the problem of the transmission of kala azar for about 10 years, the last 6 of them in attempts to transmit *Leishmania donovani* to animals and man by the bite of the sandfly *Phlebotomus argentipes*, but all in vain. The epidemic of kala-azar which had been in progress was dying down and, in

consequence, research material was becoming difficult to obtain I could see no new methods of approach to the problem, no new principles to explore and, in some depression, I recommended the closing down of the Kala-azar Commission until the next epidemic should arise Next morning the whole laboratory was in a turmoil of excitement The very last animal to be examined before the closure of the work—a Chinese hamster bitten by infected sandflies over a year before—proved to be infected In a day the clouds had rolled away, proof that transmission by the bite of the sandfly was possible had been obtained, and the way was open to extend the work until final proof of transmission to man by the bite of the sandfly was accomplished

Now all I have said so far has been almost in the nature of a sermon, and so I close this section of my address—in all reverence—in the words of a sermon known to you all in the full assurance that, if the precepts I have been advocating are faithfully followed, success will be assured The words to which I allude are, " Ask and it shall be given you , seek and ye shall find , knock and it shall be opened unto you "

Do you think from what I have said so far that the pursuit of tropical medicine as a career is all serious endeavour without lighter moments of relaxation ? Far from it get it into your heads that it is a fascinating game In following the main roads there are many side tracks up which we can wander, in relaxation, to our great advantage and entertainment Even in one's everyday work there is humour and cause for laughter, often perceived and best appreciated after the event rather than at the time

Nowadays, one hardly dares send a worker into the field unless he is provided with living conditions—houses, lights, fans and frigidaires—which we in the past considered to be the luxuries only of large centres of habitation This is all to the good when it can be done, but the enthusiast will not be dissuaded when these are not forthcoming In my own experience of field work in India we built our own mud and bamboo huts where the work happened to be These served both for temporary homes and for laboratories, in fact the latter took precedence I remember at one time in Assam, when working on kala-azar, the Governor of Assam visited my field station At the time my wife and two small children were living there with me, and His Excellency was astonished to see us all domiciled in a mud and lath hut in the compound of a good government bungalow while the whole of the latter was used as a laboratory He was reported to have said on regaining the provincial headquarters, " There were the Shortts living in mud huts in the compound of a perfectly good Government bungalow and the little Shortts running about among the bugs "

When the monsoon broke my family went up to the hills and I was left among the bugs in the mud hut My wife, in pity, left me a nice Persian rug beside my camp bed for me to step on to in the morning when I got up The

floor was mud covered with bamboo matting, and on the latter—the rug One day after a week of continuous rain, when I got up in the morning I saw to my horror grass growing through the rug! I lifted it by two corners and at the door shook it vigorously The damp had rotted it and the rug flew through the air across the compound while the corners remained in my hands!

I do not mean to infer from all this that roughing it is necessary when it can be avoided, but it is certainly a good training in teaching one to make do when all we need is not available.

I often laugh at my initiation into medical research of a young Indian medical man who afterwards became a well-known Indian malariologist. I was trying to reach a certain tea garden in the monsoon by car along a so-called *road across the rice fields. I was accompanied by three Indian workers* a Mr JAMES JOHN the late Mr C. S. SWAMINATH, my collaborator in many papers, and this young recruit whom we may call Dr X. Every few yards *we had to get out and push the car out of the mud or tie ropes round the wheels* to act as chains and renew these as they got cut on harder parts of the track. Picture to yourself our condition by the afternoon It was monsoon, it was raining a tepid flood and steamy hot. On each side of the track stretched flooded rice fields. We were all exhausted and had drunk all the liquid we had Presently Mr JAMES JOHN came and asked me for my handkerchief—already soaked with sweat and rain. He looked at it somewhat ruefully then laid it out flat on the water of the rice field and drank through it in the hope it would act as a filter! He handed it back to me with a shudder and the words, Sir I have never had to do that before." As the afternoon wore on, and it looked as if we would be benighted, it became too much for the new recruit who conjured up all sorts of horrors at the thought of a night in the jungle, and he began to weep He was sternly rebuked by Mr SWAMINATH, who said to him Now now Dr X., you needn t weep this is nothing to what you will endure in the future this is research work under the Indian Research *Fund Association." His words were true and, as I have said,* Dr X. made good

If any of you are keen on languages, there is infinite scope f r you, and *there is no doubt whatever* that at least a working knowledge of the language of the people among whom one is working is an immense help as those of you who have had to work through interpreters, *as I have at times, will agree* Besides, a knowledge of the language may be useful in other ways as what I am now going to tell you will forcibly illustrate.

Sir *RICHARD CHRISTOPHERS and I were swimming in a creek off the river* Shatt el Arab in Iraq We noticed some Arab children, boys and girls, squatting on the bank opposite to us, and these were continually being reinforced in *numbers.* We soon realized that we were not being merely admired, either for our swimming or our figures, and that there was an expectant look on some

of the faces At last, partly in exasperation and partly in curiosity, I shouted to them in Arabic, asking them what they were waiting for One of them, with almost an eager look in his eyes, said, "We're waiting to see the sharks get you!" Now, we had not expected sharks in fresh water, but shortly afterwards I realized that the danger was not so remote when, having been admitted to hospital for malaria, I had as a fellow-patient in a nearby bed a man whose leg had been badly torn by a shark at a place not far from where we had been bathing Only the presence of companions who pulled him into a boat saved his life

In speaking a little earlier, I mentioned the need for improvizing, whether it be laboratories or houses, where this is necessary and the principle applies also to many of the procedures used in the practice of medicine in places where all modern facilities are not available One quickly learns to do this, and it is surprising what results we sometimes get with what can only be described as Heath Robinson apparatus Again, I illustrate this from my own experience A great friend of mine, a tea garden doctor in Assam, was lying dangerously ill He had been diagnosed malaria but he himself thought he had kala-azar I was then working some 150 miles away, and one day got a telegram from his wife asking me in urgent terms to come and see him On arrival, I found him desperately ill and delirious, and for this reason the local medical officer, also a friend of mine, was living in the house After consultation together, we decided that the most probable diagnosis was enteric However, we had no culture media, no diagnostic sera and not even a test tube How could we confirm the diagnosis? I repaired, some miles away, to the local Indian butcher and persuaded him to kill a calf From this, with a syringe, I extracted bile from the gall bladder into a bottle On arrival back at the patient's bungalow I found the doctor had unearthed a small tube which would serve as a test tube Into this we put some of the bile We then extracted the inner tube from a tyre of the patient's bicycle and, cutting off a piece of rubber, tied it tightly over the mouth of the tube A hypodermic needle was now thrust through the rubber and left in position The tube of bile was now boiled for some time in a pan, steam from the boiling tube escaping through the hypodermic needle When the tube had cooled down, we inoculated the bile with the patient's blood and sent it off to the headquarters laboratory, together with some blood for a Widal test Strange to relate, considering the chances of contamination, the bile grew a pure culture of *Bacillus typhosus*, and the blood gave a positive Widal reaction, for the patient had been ill for 3 weeks Fortunately, although at one stage it looked as if he could not recover, wonderful nursing by his wife pulled him through

You may by now be saying it is all very well to advocate a life given to tropical medicine with its deprivations, its sometimes inevitable separations, and its comparative lack of the amenities of modern life, although many of these now reach the most remote places, but this is not a strictly true picture

To come away for a moment from the business side of our subject, let us consider but very shortly some of the amenities. We do not work all the time we also play Not only do we thus amuse ourselves but we actually have a wider choice of amusements, at least athletic amusements, than in the centre of civilization. Many things become possible and even easy which elsewhere can only be done by the very rich—now of course an extinct class in Great Britain. I refer to such things as polo, big game shooting and even such apparently unlikely activities as skiing on the equator

As residents in the country where they exist you can shoot elephants, lions, tigers, leopards, bear bison, buffalo and what you will at moderate cost or if you have no desire to kill for tangible trophies, you can photograph these same things and still have something to jog your memory in later years and attest to your veracity

But what is the other side of the picture? What are the positive gains to ourselves and to our fellow men which may accrue? This question can only be answered by considering the many problems awaiting solution by you younger men problems some of which have been only half solved by workers of previous generations and require completion, some of which have been little more than formulated and some which as yet we are not even aware of This is equivalent to saying that there is no end to the asking of questions, and however much one generation may elucidate it is only a very small part of the structure of knowledge it is our privilege to explore and from which we must try to wrest the secrets which nature always so jealously guards, while we need not fear that in this great quest there will ever be any finality when nothing more remains to discover Even when we have discovered the immediate causes of disease the ultimate causes will still be eluding us. As Walt Whitman has said, from every fruition of success, however full, comes forth something to make a greater struggle necessary

In mentioning even a few only of the endless problems awaiting solution, you will realize how widely the net may be cast and that in it are treasures to satisfy every bent. Nor is it necessary to plough a lonely furrow for many problems require the co-ordination of different lines of attack converging on a given objective Take for instance, a syndrome such as sprue. It is still a very mysterious condition. Its curious distribution, its pathology its physiology its bacteriology and its bio-chemistry open a field for combined attack from many angles. Think of the joy of directing a successful attack on its hitherto inscrutable obscurity of lighting up its dark places and so bringing its treatment within the realm of scientifically applied measures based on an adequate knowledge of its aetiology! Here is an opportunity for pathologists, physiologists, bacteriologists, bio-chemists and chemotherapists to play together as a team. It could be done -therefore why not do it?

What of that greatest of all killers of the human race—malaria? Great

strides have been made in our knowledge of malaria and how to cure and prevent it But do you think that DDT residual spraying, the various repellents and the new antimalarial drugs in our armamentarium have fired the last shot and given its quietus to malaria ? That result may come some day, but the day is not yet We are still a long way from the ideal specific drug or drugs, and if such a drug were found tomorrow we are still a long way from ensuring its universal distribution and use Why is it that so much research is going on in connection with the prevention of malaria from many angles ? It is because much has still to be learned and only those actively and practically engaged on the work realize how much this is We still do not know how much harm, as well as good, we may be doing by the widespread use of DDT It must not be forgotten that many forms of insect life destroyed by DDT play their part in the ecology of nature in any given area and that the state of ecological equilibrium has been built up over periods geological in their duration If we suddenly upset this equilibrium only time will reveal the result, but it is a fruitful field for study The very malaria parasite which it is the object of malariologists to abolish has its place in the ecological picture and who knows whether, if they succeed in driving out this devil completely, seven other devils may not take its place !

But even if we do not wholly exterminate the malaria parasite there are many of the intimacies of its private life we may still pry into to satisfy our morbid curiosity The adjective " morbid " is probably correct when it refers to the curious prying of the so-called highest form of life into the inmost privacies of one of the lowest forms of life Why does the malaria parasite find one kind of mosquito a congenial host and another kind wholly inimical ? Why have we not yet discovered the answer to this problem which is common ground to entomologist and protozoologist ? Why does the erythrocyte, which is host to the benign tertian malarial parasite, develop freckles, more usually known in this connection as Schuffner's dots ? Is there such a thing as malarial toxin, as some of the findings in the pathology of malaria would appear to indicate ? If so, why have the biochemists not isolated or even found it ? I blush to delve further, but those with less fastidious minds could think of many other intimate and hitherto unrevealed details of its private life, knowledge of which the malaria parasite will no doubt do its utmost to maintain inviolate

And what of blackwater fever ? Almost the only established fact about this is that the syndrome is connected with malarial infections Why does the malarial subject suddenly, and often without warning, suffer the loss of a large proportion of his erythrocytes and arrive, in a matter of hours, at death's door ? What has made his red cells so dangerously vulnerable that some trigger action releases, in a moment, the destroying force Could the catastrophe have been prevented ? Once it has happened is there any effective means of retrieving the position ? None of these questions can yet be adequately answered, and they are a challenge to you all but, perhaps, especially to the

physiologists. When they are answered we may arrive at a knowledge of rational methods of prevention and if these are neglected by the ignorant or ignored by the careless, *of cure*.

Now let us consider the problem of African trypanosomiasis. *Or do you think it is no longer a problem?* Do you think that, here again the modern insecticides used to attack the vector tsetse flies will supply the complete answer to prevention at least? *Well it may be so but the cautious will defer judgement* in line with the principles I enunciated at the commencement of this address. The fact remains that numerous instances could be quoted showing that *outbreaks of trypanosomiasis due to Trypanosoma evansi and T. congolense* have occurred in the apparent absence of tsetse flies. In these cases transmission, normally achieved by the agency of tsetse flies, was probably effected by interrupted feeding of various biting flies such as tabanids and stomoxys, known to be present in very large numbers. But, apart from the disease and its transmission there is still a great deal to be learned about the trypanosome itself. I believe we still have a very imperfect knowledge of its life history in the vertebrate host, and this is a problem requiring immediate solution. Some are working upon it, but if more were to do so the answer might come sooner. This is only one aspect. The classification of trypanosomes is still in a state almost chaotic, especially when we consider the wide phylogenetic range occupied by the genus in the vertebrate kingdom—mammals, birds, reptiles, amphibia and fishes—and this chaos will not be changed to order until we know enough of the full life histories of a sufficient number of species to give us data for a rational classification.

Then again, there has recently been described a new trypanosome of man in S. America—*T. rangeli*. Is this a cause of disease or is it an accidental and evanescent visitor in man?

Let us turn for a moment to a closely related genus of parasite which occurs as an agent of disease in man and animals in every continent except possibly *Australia. I refer to the genus Leishmania, the cause of kala-azar* oriental sore and espundia. We know the vector in the case of Indian kala azar but in the case of the visceral disease elsewhere in the world the alleged vectors have been incriminated *if at all, only on epidemiological grounds and* unequivocal scientific delimitation of vectors has still to be achieved. The same remarks would apply to the vectors of the various dermal lesions produced by *Leishmania* in different parts of *the world, although verification of the* vectors of oriental sore in the Mediterranean area now require only the dotting of the "I's" completely to satisfy the requirements of scientific proof.

A good deal of attention has lately been fixed upon toxoplasmosis. *Now we know the organism responsible for this condition the Toxoplasma* but we have no idea as to the method of infection, although the parasite is found in mammals, birds and reptiles. The human disease is most often manifested

in very young children who show encephalitic symptoms shortly after birth and who are already infected *in utero* In many or most cases the mother does not and never has shown any symptoms of infection yet, when her blood is tested, it can be shown to contain antibodies to *Toxoplasma* Here is an interesting field for research—although not strictly speaking in tropical medicine—because we do not as yet know even the taxonomic status of *Toxoplasma*, we do not know whether to call it an animal or a vegetable and we know nothing of its life cycle or whether it has any free-living existence

Let us leave animals for the time and consider the plants Tropical mycology—what an unexplored and labyrinthine jungle it conjures up to those of us who have lived for any time in the tropics and seen the varied manifestations of mycotic diseases in man and animals This is probably one of the least explored branches of tropical medicine and therefore a fruitful field for endeavour The majority of the diseases caused by the mycetozoa do not kill and therefore are less spectacular than the killing diseases such as cholera and plague, to mention only two, but the suffering and disfigurement they cause, their ubiquity in the tropics, their relative refractoriness to treatment and the lack of precise knowledge about them make them one of the major medical problems of the tropics Here is a field where new knowledge is to be acquired as soon as the study is taken up because at present the ground is almost untrodden

Now let us consider an example of helminthic diseases—onchocerciasis This is caused by a filarial worm of the genus *Onchocerca* The condition is found in man in Africa and Central America It is responsible for nodules in the sub-cutaneous tissues and for eye lesions which may even lead to blindness Treatment with the newer anti-filarial drugs is not wholly satisfactory The disease is spread by flies of the genus *Simulium* In Kenya, where the local vector is *S neavei*, no one has yet been able to find the breeding place of this insect

Up to this point I have not touched upon the virus diseases, and we may now consider these Yellow fever is a virus disease in connection with which research work in recent years has revolutionized our ideas of its distribution, its vectors, its animal reservoirs and its epidemiology and endemiology in general Work of vital and fascinating interest is even now in progress and in this you can take a part for there is still a multitude of lines of enquiry awaiting workers with imagination and application It offers a wonderful tangle to be unravelled from the intimate association of virus, mosquito, man, monkey and, possibly, other animals

What of dengue fever? This occurs sporadically as well as in severe epidemic form, the latter often in ports, such as the epidemics which have occurred in Athens and in Calcutta among other places What happens to the virus in the intervals between epidemics? Are there human carriers or is there some animal reservoir? If the latter, why does the virus suddenly concentrate on the human host?

While on the subject of virus diseases, I should draw attention to a field which is still comparatively virgin ground and where every step taken should carry the thrill felt by an explorer entering territory never before traversed. I refer here to the viral encephalitides, the series of virus diseases—probably mostly insect borne—causing encephalitis in man and animals in different parts of the world. Among these to name some which have been partially studied, are St. Louis encephalitis Japanese B encephalitis, Australian X disease, Western equine encephalitis, Eastern equine encephalitis, Venezuelan equine encephalitis and Russian far east encephalitis. To come to infections in the tropics or subtropics, we have West Nile virus Bwamba fever in Uganda Semliki forest virus and Bunyamwera virus both in Uganda, and several others I have not mentioned Not all of these occur in tropical countries but some do as the names obviously imply and there are here, among these various conditions, great lacunae in our knowledge waiting to be filled. The general pathological findings in the viral encephalitides are dependent on the fact that viruses are obligate dwellers in intra-cellular habitats and they produce their first effects on the cells they inhabit, in the cases I am considering usually the cells of the central nervous system. The symptoms will necessarily be related to the parts of the central nervous system involved, but this will often not be enough to delimit a virus and laboratory investigation will be necessary A variety of arthropods are suspect as vectors and various animals as reservoirs of these viruses. This means a cycle of reservoir arthropod man, but the proof of these assumptions by scientifically controlled experiments has still to be demonstrated. The virologist the entomologist and the epidemiologist, are the workers who should wield the spades to fill in the gaps.

So far I have not mentioned one of the most important tropical diseases —amoebic dysentery and its complications such as liver abscess. What are the factors influencing the onset of amoebic hepatitis and its more ad anced stage of liver abscess? Why is it that while people in temperate climes such as Great Britain may harbour the parasite, *Entamoeba histolytica*, yet true amoebic dysentery is extremely rare and liver abscess almost unknown? Are nutritional factors invol ed, or are there pathogenic and non pathogenic strains or even distinct species of amoebae? Here is a truly intricate subject for research which might well employ an *ad hoc* team of clinicians, nutrition experts and protozoologists who would have t work in the closest association.

Earlier in my address I mentioned the importance of nutritional research in the tropics. This subject is assuming an ever increasing prominence and none can deny its paramount importance in considering any schemes of development not only in our colonial empire to mention the aspect which most closely concerns our own domestic outlook but in all countries of the tropical world It seems paradoxical that in many cases the peoples who live on a soil and in a climate which produce potential sources of food, animal and vegetable with a luxuriance not seen in temperate climes and where the

minimum of effort over a part only of the year will yield enough food to live upon for the rest of the year, should suffer from deficiencies of diet, but so it is in many places Many of these deficiencies are known to have been extensively studied, but only those engaged on such work know how small our store of established knowledge still is in comparison with that it is essential to acquire before remedial measures can first be formulated on established facts and then be applied in a manner acceptable to the people concerned The latter part of the problem is not the least important as it may have to take into consideration religious, social and racial prejudices which only education, time and successful demonstration of the benefits elsewhere will remove In some cases these deficiencies may be the actual cause of the abnormal conditions they precipitate, while in other cases cause and effect may be less clearly cut and the deficiencies may aggravate diseases due to other causes In still other cases, the converse may hold and the abnormal states may be the result, not of deficiencies, but of nocuous substances present in food or water This condition is seen in the case of endemic fluorosis in Madras province where, in certain districts, a large percentage of the population, and also of the cattle, are affected with bony growths on the long bones and ribs, and complete rigidity of the spine due to intervertebral ossification leading to great incapacity and, in extreme cases, to death from intercurrent disease, all due to excessive amounts of fluorine in the natural sources of drinking water No practical solution has yet been found for this problem, although such a solution would be to the incalculable benefit of the whole population of the district

In denoting the various problems requiring solution, I have dealt chiefly with those conditions resulting from the action of agents of disease, but there is one very important line of investigation in which knowledge is required most urgently although no disease process is involved I refer to the special physiological functioning of the body in tropical climates This is a very large subject on which much has been done but more remains to be done It should open up a highly interesting field for study to the worker interested in physiology, and the knowledge gained will have important and immediate applications in many directions As the tropics are opened out for habitation by non-indigenous peoples, and remember there are at present vast uninhabited or extremely sparsely-inhabitated areas, a knowledge of physiological variations in hot and humid and hot and dry climates will be of great importance in making life not only supportable but pleasant for such newcomers

Such a process of opening out a country will necessarily involve large-scale employment of labour, either in industry or agriculture or both, and physiological problems will be involved in the general care, housing, feeding and conditions of work and play of these people A fore-knowledge of what to do will be repaid a hundredfold in the health and happiness of the newly-opened territories

One aspect of careers in tropical medicine I have not yet touched upon

is the possibility when a reputation has been established, of becoming a teacher of the subject. There are openings, both in temperate climes and in the tropics themselves, for such activities and for those with a bent in this direction there could be no better outlet than to pass on the knowledge they have acquired to the coming generation of workers. Teaching can be a very humdrum business, both for teacher and student, and the really inspired teacher comes seldom—for the fire must be there—but when found, is a gift to be cherished, for he can pass on his inspiration, which may be even greater than his knowledge, to generations of students

So far I have been trying to draw for you a picture of what to expect if you take up a career in tropical medicine, of its fascination as a very young sister of medicine in general, of the possible hardships and deprivations, but also of the abundant and satisfying rewards. In speaking of these rewards, here, at least, is a work with some incentive, that rare commodity in these days of levelling, when all men are proved equal by mere reiterated assertion, where man-made laws attempt to frustrate the immutable laws of nature and the rewards of labour are no longer ours to enjoy Thank heavens, the rewards I refer to are intangible and beyond the reach of the levellers, the planners and the income tax collectors. The worker who has succeeded in laying bare some of the secrets of nature and thereby possibly benefited his fellow men, has his own personal reward in the shape of achievement, and who shall say that he has not proved the fallacies of the levellers by the mere fact of rising above mediocrity

But having drawn the picture, you will want to know how to achieve the aim of a career in tropical medicine and, as I wish to keep my address on a practical not it is only fair that I should give you some guidance. On account of the speed of modern transport, the world is now a small place, and this alone has enhanced the importance of tropical medicine. As an empire, and I am not afraid to use the word which we, as a people, have made honourable, and as a member of a world wide commonwealth of nations, our commitments are also world-wide and so our armed services penetrate to every quarter of the globe. This necessitates a similar penetration by their medical services, so this is a first suggestion as to a means of entry to the practice of tropical medicine. How fruitful a method this is could readily be exemplified by names such as ROSS, GORGAS, LEISHMAN, JAMES, CHRISTOPHERS, SINTON and many others I could mention. If any of you should decide on this method, do not be discouraged if you are not immediately asked to undertake some difficult research problem in the tropics. You will only become worthy after enduring many disciplines which may seem futile at the time but which are inescapable in any service and the worth of which is often only recognized in later life. Discipline, in any of its connotations, is a very valuable thing and indispensable in truly civilized communities. For those of you who are not fired with martial

ardour, love of the sea, or going to strange places by air, there is the colonial service The widespread nature of the Empire gives you ample scope here and, to a large extent, you can choose your sphere of work not only as regards territory but as regards the branch of tropical medicine you wish to take up —again provided you are prepared to undergo the preliminary disciplines of the early stages of your career There are ample opportunities for work on the clinical, laboratory and public health aspects and ability and initiative will reap their reward

In the case of those entering the colonial service, it used to be customary to take the Diploma in Tropical Medicine and Hygiene before going out to the tropics When there arose an acute shortage of recruits for the service this was no longer possible and those entering the service were sent out without preliminary training and had to learn what they could in the actual practice of the profession Such men could later come back and take the Diploma in this country, having already gained some experience in the tropics It is at least open to consideration which method is the better, but my own inclination would be to give the man instruction in tropical medicine and let him take his Diploma before going to the tropics He would thus gain his experience with at least some theoretical background of knowledge and could, when he returned, and if he was specially interested in some particular aspect of tropical medicine, concentrate on advanced studies on that aspect However, I am open minded on the subject, and perhaps this question could best be left to the Colonial Office, with its special knowledge of the conditions of service

Some of you may wish to work completely untrammelled by the rules and regulations of services, whether armed or civil, and for you there are other openings to consider Many of our major industrial undertakings, still outside the reach of the planners' grasp, such as the oil industry, the tea and coffee industries, the rubber industry and others less well known, have large interests in tropical countries and maintain large staffs there Most of these undertakings now maintain their staff under very favourable conditions as regards pay and general amenities, and among the latter are excellent medical services, the hospitals of which are quite often better equipped than those of official Government organizations

This does not exhaust the variety of openings for work in tropical medicine, but I have said enough to indicate that for those who will, the opportunity is there and the work is worth while

One thing, at least, you can look forward to and that is that you need never fear a stale humdrumness in life, where day in day out and year in year out you follow a few stereotyped activities repetitively performed for ever and ever In the life I am advocating you may, in your time, be many things You may on Monday have to start building a bridge, on Tuesday you may

be performing an abdominal operation by candlelight on Wednesday you may spend half the day burning an anthrax carcase on Thursday you may be settling a quarrel between two sets of villagers on Friday you may be taking over as a temporary measure the duties of Governor of a province on Saturday you may actually be doing something connected with tropical medicine and on Sunday you may be taking a church service. All but two of these duties have fallen to my own lot at different times.

Well, ladies and gentlemen, I have occupied your time long enough, and I only wish that someone more eloquent and more persuasive than I could have delivered my address and made the plea which I have made for the study of tropical medicine as a worth-while career In the earlier part of my address I advocated courage, and I now close on the same note. We live in great and stirring times. The days of Elizabethan England, the period of the Napoleonic wars and the time of British Empire building to mention only three recent phases in our island history pale into insignificance in the dazzling glare which lights events in our own time. As with the times, so with the men. As current events are on an altogether mightier scale than those of past times, so the actors in them, both malevolent and beneficent, are giants by comparison, and you yourselves can easily give them names. Likewise, the responsibilities shouldered by us all are greater in proportion to the increasing numbers of people affected by events in a world rapidly contracting in virtue of modern speed of transportation, and this calls for the highest qualities of faithfulness, confidence, clear vision, far-sightedness and ruthless resolution if world problems are to be settled in a way to allow man to pursue his destiny in peace and happiness. I do not wish to enlarge on this and have only made these comments to emphasize that if we live in difficult times, they are great and glorious times, giving the opportunity to do greater things to remedy greater evils, and it is well we should realize our good fortune and be worthy of it.

Now is the time for British youth to be adventurous, to be prepared to welcome and challenge present world problems, and one of the most important of these for our country is to fill the unoccupied spaces in the Empire, if possible with people of our own blood and with people from our Commonwealth of Nations the daughter countries which are now vying with the mother country in their influence on world affairs. Even if there were no worthier motives for peopling the waste spaces of the earth, surely the recent world war supplied evidence enough of the danger to their peoples of thinly inhabited cou tries. The quickest way to achieve this result is to get rid of the causes which make these places waste lands for man, the agents of disease in man and his domestic animals, and so to create the conditions which will make them suitable and fit to produce food and breed men. Be up and doing be prepared to venture when opportunity beckons. We still have the brains, let us show that we also have the guts!

The Society's Thanks and Congratulations to the President

Sir George McRobert I rise to thank our President on behalf of the Society for his able and stimulating address He has reminded us that the tropics still provide the keen and adventurous worker with fruitful fields of labour of absorbing interest

Many people today feel that political and administrative rearrangements among tropical lands have lessened the opportunities for doctors from this country That may be so, but it is probable that with the intensive drive for improvements in tropical Africa and in the Caribbean a large number of European workers will be needed there for many years Now, more than ever before, the tropical schools in Britain will be required to act as headquarters of teaching, of expeditionary research forces and of advanced research in tropical medicine and hygiene

Our American friends and rivals in tropical medicine, as in other fields of scientific work, have since the end of the war been stretching out kindly and welcoming hands to many students from the Commonwealth and from those Eastern countries formerly closely bound up with our fortunes With the revaluation of the pound, this flow of students across the Atlantic is bound to lessen, and it is urgently necessary for us here to make certain that a warm welcome is accorded to those who would otherwise have crossed the Atlantic and to ensure that for Australasian, Indian, Pakistan and South African students, as well as Burmese, Chinese, Egyptian and other nationals, Britain shall continue to provide instruction and help for those who wish to adopt tropical medicine as a career

Throughout his long service in India Henry Shortt was known as a bold, enterprising and successful sportsman in all branches of the chase In the clubs they tell of gigantic tuskers, man-eating tigers and outsize bison which have fallen to his rifle His skill with the rod—from mahseer to trout—has been the despair of rival sportsmen In the field of microbiology he has successfully stalked *Babesia canis*, *Leishmania donovani*, *Plasmodium vivax*, and the viruses of dengue and sandfly fevers He has, indeed, been a mighty hunter

I have the great privilege of making the first announcement of the complete success of his most recent hunt—the quarry being *Plasmodium falciparum* During the past 10 days, Professor Shortt, in close collaboration with Dr Hamilton Fairley, on whose pioneer work at Cairns the outcome of war in the Pacific depended, has conducted an experiment on a healthy human volunteer

Using his technique of mass invasion of the body by many millions of sporozoites introduced by the bites of hundreds of highly infective mosquitoes (*Anopheles maculipennis* var *atroparvus*) on 3 successive days SHORTT has been able to demonstrate in the parenchymatous cells of a piece of liver removed on the sixth day after the first infective bites, three successive stages in the development of the pre-erythrocytic form of *Plasmodium falciparum*—the largest single form so far seen apparently containing over 30 000 merozoites ready to rupture into the liver sinusoids. I am requested to mention the valuable part played in the work by Mr P G SHUTE, of the Ministry of Health Malaria Laboratory at Horton. In breeding feeding and tending mosquitoes he has no equal and his knowledge of their habits in the laboratory is unsurpassed. Sir GORDON COVELL obtained the strain of *P falciparum* from Rumania he has given most valuable advice and co-operation. Dr W D NICOL, of Horton was as helpful as ever in providing clinical cases. Mr NAUNTON MORGAN performed the biopsy cyclopropane anaesthesia was administered by Dr HEWER.

Tribute must be paid to the anonymous, brave and adventurous healthy human volunteer who offered himself for the experiment without thought of reward.

This timely and triumphant outcome of our President's most recent researches enables us, as a Society to combine thanks for his masterly address with congratulations to himself and his team of colleagues.

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COMMUNICATIONS.

MALARIA AMONG PRISONERS OF WAR IN SIAM ("F" FORCE) *

BY

T WILSON, M B , D P H ,
Health Officer, Malayan Medical Service,

AND

J A REID, M SC ,
Entomologist, Institute for Medical Research, Malaya

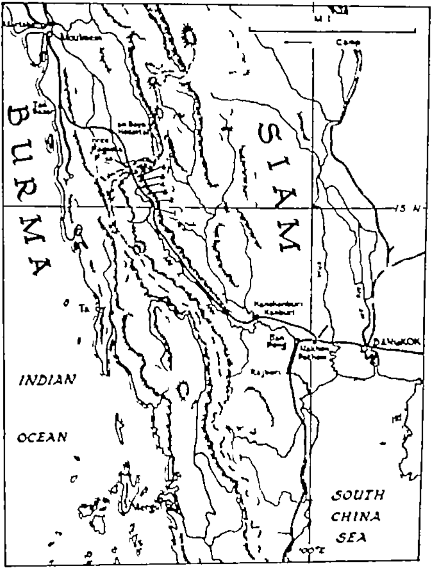
In April, 1943, the Japanese sent a party of 7,000 British and Australian prisoners of war to Siam from the main camp at Changi on Singapore Island, to work as labourers on the construction of a section of the railway then being built to link Bangkok with Moulmein in Burma Known as "F" force, to distinguish it from other parties despatched before and after it, this party was given the task of making the bridges, embankments and cuttings for the most northerly section of the line in Siam It was a section roughly 45 miles long, commencing at an altitude of about 3,000 feet at the Three Pagodas pass on the water-shed dividing Burma from Siam, and running south-east in the valley of the Me Nam Kwa Noi near its head waters (Map)

We were attached to the force as advisers on malaria and nutrition We possessed the only available microscope, and an equally precious small stock of stain These had to be carried from camp to camp in the microscope box, slung between two of us on a bamboo pole , thus microscopic diagnosis of malaria was confined to those camps that we were allowed to visit, and to the

* We gratefully acknowledge the sterling work of L /Corporal K ANDERSON and Pte J R SHARP, of the 2/10th Field Ambulance, Australian Army Medical Corps, who prepared and stained blood films, kept records, and assisted in carrying the microscope, and in many other ways

They joined us at Camp 1 and stayed with us thereafter Without their loyal and cheerful help, our work could not have been accomplished We also wish to thank Lieut - Colonel J HUSTON, R A M C , Senior Medical Officer of "F" force, and the medical officers and many other officers and men of the force, British and Australian, for their support and comradeship

We are indebted to Brigadier J BENNET for permission to publish this account , and to Mr YAP LOY FONG, of the Institute for Medical Research, Kuala Lumpur, for his help in drawing the figures and lettering the map



Camp 1 Shimo Nieke
Camp 2, Nieke
Camp 3 Shimo Sonkurai
Camp 4 Sonkurai
Camp 5 Kami Sonkurai
Camp 6 Changaraya.

periods of our visits Our figures are therefore only a small sample of the total malaria experienced by "F" force, but they have the merit of being the only exact figures in existence We have thought it worth while to record them, in spite of their limited scope, as they differ in some respects from the few accounts of malaria and anophelines in Siam which we have been able to consult

MALARIA BEFORE LEAVING SINGAPORE AND ON RETURN

The incidence of malaria during the brief Malayan campaign had been low Transmission in Changi camp had averaged only 1 4 fresh infections per thousand men per month over the 11 months ending February, 1943, and the other Singapore camps were not much more malarious at that time A somewhat hurried last-minute blood examination of the whole 7,000 men, made on the orders of the Japanese, showed that they were almost malaria-free just before their departure for Siam in April, 1943, they must be regarded as forming an uninfected and non-immune population

Eight months later, the picture had changed completely In December, 1943, 3,122 of these men returned to Changi A complete blood survey in the following week revealed 801 malaria parasite carriers, a parasite rate of 25 7 per cent Since many men were still under treatment, this figure is artificially low, but allowing for this it is thought to be consonant with the estimate, based on sickness records, that at least three-quarters of the force had suffered from malaria Many had had it frequently, and it was common to meet men with histories of 10 to 16 attacks during their 8 months' stay in Siam In the next 7 weeks, these 3,122 men produced 2,942 separate attacks of malaria, an attack rate of 18 8 per thousand men per day By this time, very few men had no history of recent fever, so that the great bulk of these attacks had to be classified as relapses or reinfections, hereafter grouped together as "secondary" attacks

The next section of this paper is a description of the conditions encountered in Siam which were responsible for this thoroughgoing transformation of a malaria-free community into a very heavily infected one

MALARIA IN SIAM

The general conditions experienced by the force and the treatment meted out to it by the Japanese have been described elsewhere (REID and WILSON, 1947)* and need not be repeated here †

* "Report on nutrition, and discussion of the main causes of death, 'F' force, Thailand" *J R Army med Cps* 89, 149 Through chance seniority in army rank my name appears as senior author of this paper, which is almost entirely the work of T W The title is a little misleading, for in editing the paper, which is extracted from a report made to our medical headquarters in Changi, the discussion on the causes of death has been largely omitted —J A R

† For a full description of the life of prisoners of war in Siam the reader is referred to such books as "Behind Bamboo," by ROHAN D RIVETT (Sydney Angus and Robertson 1946)

The Train Journey.—The negligible amount of malaria in Changi before leaving for Siam has already been mentioned. The train journey to Siam a distance of about 1,200 miles, took 4 to 5 days. We travelled by day and by night, living and sleeping in steel goods wagons, and halting for irregular intervals at various wayside stations. It is uncertain how many infections were acquired on this journey probably only a few

The March.—By contrast, there is no doubt that many infections were contracted during the march from Ban Pong, the detraining point on the main line railway to the fixed camps some 170 to 200 miles north west near the Burma Siam border This march lasted about 3 weeks, and was made by night, the route lying up the valley of the Me Nam Kwa Noi. There was every opportunity for infection. One slept in the open by the wayside for a few hours in the middle of each night, often in close proximity to Siamese villages or earlier-established prisoner-of war camps where as we later discovered, malaria had been prevalent. As a result, many men developed an attack of malaria within a few days of arriving at their camp

The Camps.—The force was split up into a number of working parties camped a few miles apart along the projected railway track. The five camps which we were allowed to visit were strung out over a distance of about 15 miles. (See map) Starting work on 24th May 1943 in the southernmost one, Camp 1 (Shimo Nieke), where we had ended our march 2 days before, we arrived in Camp 5 (Kami Sonkurai) on 1st August, and remained there until its evacuation at the end of November We were unable to visit Camp 6, which was disbanded a few days after we reached Camp 5, and we never managed to contact a group of camps further south at Konkoita, or a so-called hospital camp at Tanbaya in Burma, which had been filled with the chronic invalids from the working camps.

Topography Vegetation and Climate.—These camps lay at an levation of 2,000 to 2,500 feet, the Three Pagodas Pass itself being about 3 000 feet. They were situated in valley beds of widths varying from a mile or more to a few hundred yards. Close to Camps 2 and 3 there were patches of abandoned rice field, but the other camps were merely small freshly made clearings in the jungle. The hill behind Camp 5 ended in a limestone pinnacle which we occasionally climbed surreptitiously From this vantage point mile upon mile of jungle-covered hills could be seen reaching away t the limit of vision in all directions. Inhabitants were few although there was one small village Nieke a few miles west of Camp 2.

The vegetation was monsoon rain forest consisting of a mixture of deciduous and evergreen trees of varying sizes occasional big trees f well over 100 feet in height stood out above the general canopy which was at perhaps 50 to 80 feet. The big trees in particular carried a heavy load of lianes and there was a dense undergrowth of large and small bamboos with a carpet of plants of the ginger family which flowered profusely at the beginning of the rains.

The heavy rains of the south-west monsoon commenced in the second week of May whilst the march from Ban Pong was still in progress, and continued with little intermission until the end of September Thereafter the days were hot and dry with bright sunshine, and the nights increasingly cold, until our departure from the area in November Only a few thunderstorms interrupted this dry weather, and the country rapidly assumed its dry season aspect

BLOOD EXAMINATIONS

(a) *Technique and Standards*

Technique —Thick blood films were used throughout , we had no materials for thin film staining Field's rapid stain (FIELD, 1941), which proved invaluable under prisoner-of-war conditions, was used from 24th May until 20th August, when an accident deprived us of the last few drops Blood examinations ceased for the next 7 weeks until mid-October, when we obtained some Japanese giemsa, as explained later, and were able to use it for the remaining 38 days until the camp dispersed Field's stain was again available at Kanburi hospital, and after our return to Changi

Negative Films —A film was reported "negative" if no parasites were found in 100 thick-film fields After a negative first film, repeat films were taken if requested by the medical officer Ninety-seven per cent of all positives were found on examination of the first film

Fresh Infections —A man was regarded as having a fresh infection if he gave a history of freedom from fever for the past 12 months

Local Infections —Fresh infections occurring in men who had lived in a particular camp for 15 days or more before the onset of symptoms, were considered as local infections

Species Unidentified —This diagnosis covers all infections with less than one parasite per thick-film field in which the species could not be identified with reasonable certainty It was not possible to take repeat films from all such cases, nor could we check our results with thin films

(b) *Table of Results* (Table I)

Malaria at each Camp — Blood examinations at Camps 1 and 2, and enquiries made subsequently at other camps, gave the same general picture of numbers of men going down with fever a few days after arrival, obviously a result of infection acquired during the march Subsequent happenings varied at each camp

Camp 1 (*Shimo Nieke*) —This, the first camp at which we were able to examine bloods, was full of the sicker men left behind by the various parties passing through on their way farther north Of the films containing parasites, 84 per cent (103/122) were fresh infections , most of these were from men already sick on arrival, or who went sick very shortly afterwards. We were

TABLE I

BLOOD EXAMINATIONS IN "F" FORCE SIAM—1943

Date	Camp.	Number of day examination.	Average camp strength.	Total infections. P. vivax.	P. falciparum.	Mixed.	P. malariae.	Species undetermined.	Total.	Fresh infections. P. vivax.	P. falciparum.	Mixed.	P. malariae.	Species undetermined.	Total.	Per cent. fresh infections.	Total negatives.	Total films examined.	Per cent. positive.
14 May to 5 June	No. 1	13	About 600	75	39	—	—	9	123	65	31	—	—	7	103	84	103	226	54
12 to 30 June	No. 2	19	1 025 from 16th June	292	117	8	1	45	463	203	78	6	—	31	318	69	205	668	69
1 to 7 July	No. 2	7	1 018	93	22	3	—	2	120	40	9	1	—	2	52	40	61	181	66
8 to 20 Aug.	No. 2 men done at No. 5	(15)	370	46	8	—	—	7	61	13	3	—	—	2	18	30	104	165	37
19 to 21 July	No. 3	3	1 650	99	13	4	—	4	120	9	1	1	—	—	11	8	62	182	66
8 to 20 Aug.	No. 3 men done at No. 5	(15)	810	89	7	—	—	8	104	3	1	—	—	1	5	5	121	225	46
23 to 31 July	No. 4	10	1 300	29	11	—	—	5	45	9	4	—	—	1	14	31	115	160	28
4 to 19 Aug.	No. 4 men done at No. 5	(15)	330	53	23	3	1	4	84	16	9	2	—	3	28	33	61	145	59
2 to 20 Aug.	No. 5 original occupants	10	375	[illegible]	7	—	—	4	33	3	—	—	—	—	3	9	85	120	29
18 to 31 Oct.	No. 5	14	1 450	184	12	1	—	5	202	22	1	—	—	1	24	17	153	354	57
1 to 4 Nov.	No. 5	24	1 370	298	23	3	—	6	330	31	3	—	—	1	35	11	209	539	61
9 to 18 Dec.	Kanburi Hospital	7	1,000	166	23	1	1	2	193	19	4	—	—	1	[illegible]	13	233	426	45
Totals		118		1 448	314	23	3	101	1 889	441	142	10	—	52	645	34	1,811	3,400	56

unable to make any larval surveys here, and within a fortnight we were transferred, along with most of the other occupants, to Camp 2

Camp 2 (*Nieke*) —Fresh infections formed 69 per cent (318/463) of the films found positive here during June Less than a quarter of these had been in the camp long enough to have acquired their infection locally, the majority, therefore, had been infected somewhere else along the route There were just over 1,000 men in the camp, of whom about half come from Camp 1, and the rest had just arrived from farther south

In the first week of July the attack rate fell slightly, and fresh infections were 40 per cent (52/130) These were considered to be local infections, as by then everyone had been resident at the camp for 15 days or more Unfortunately our records for the next 10 days, 8th to 17th July, were lost

At this time it is estimated that the malaria transmission rate was high enough to infect at least one-third of the population per month, but the true situation in this respect was becoming obscured by the rapidly rising proportion of men with repeated attacks Indeed, from now on so many men gave a history of recent fever, that it became increasingly difficult to judge just how malarious any camp site still was

Close to this camp, larvae of *Anopheles maculatus* were plentiful, breeding in large areas of grassy seepage at the edge of old rice fields *A minimus* larvae were also present in small numbers in the same place, and in sections of a grassy edged stream (Table II)

Camp 3 (*Shimo Sonkurai*) —The senior medical officer here stated that the number of fresh fever cases had increased sharply about 2 weeks after the camp had been occupied, and continued to occur at such a rate that when we arrived there 2 months later, on 18th July, he considered that 80 to 90 per cent of the 1,850 men had already had clinical malaria We spent only 3 days here before being moved on by Japanese orders, the malaria rate was still high, and only 8 per cent (11/120) were fresh infections

Another abandoned rice field lay close to this camp also, and within a few yards of the huts there was very heavy breeding of *A maculatus* along a hill-foot seepage, with *A minimus* in a few places at the edge of the field

Camp 4 (*Sonkurai*) —There had been much less clinical malaria here than at Camp 3 The attack rate was low during our 10 days' stay, 22nd to 31st July, and 31 per cent (14/45) were fresh infections The population of the camp was 1,300 men

Larvae of *A maculatus* were found in small numbers along the course of some streams where jungle had been felled and cleared

Camp 5 (*Kami Sonkurai*) —There had been a considerable amount of clinical malaria before our arrival on 1st August, but during August the attack rate was comparatively low among the 375 original inhabitants, fresh infections were only 9 per cent (3/35) A few days after us, parties were sent here from Camps 2, 3 and 6, which were being vacated, thus raising the population of Camp 5 to 1,680

On 20th August the small remaining stock of Field's stain was accidentally spilled, and blood examinations ceased. The fever rate was fairly low for the next 7 weeks, perhaps a result of the somewhat irregular quinine suppression which had been started on 11th August.

Early in October the Japanese demanded a malaria parasite survey of the whole camp and thus enabled us to obtain from them some Japanese manufactured giemsa stain, previous requests for which had been unsuccessful. Empty oval herring tins, with two strips of plasticine along the bottom to grip the edges of the slides, made useful staining troughs: by taking four films on each slide and squeezing 50 slides into each tin it was possible to stain 200 films at a time. By economizing in this way sufficient surplus giemsa was accumulated to resume routine blood examination of all fever cases from mid October until the camp was finally closed.

This parasite survey was very hurried: to finish it in the allotted time 300 films had to be examined daily for 5 consecutive days, and we were obliged to regard a film as negative if nothing had been found in 25 thick film fields instead of the usual 100 fields. Many men were receiving treatment for clinical malaria, and others had been given quinine as a suppressive up till 10 days before the survey. These factors would all tend to diminish the proportion of positives. Results were

Period 12th to 18th October 1943

Total examined	1,505	Negative (25 fields)	1,385
Malaria parasite rate	8%	Positive (*P. vivax* 95, *P. falciparum* 25)	120

In the ensuing 38 days, 18th October to 24th November, the malaria attack rate was fairly high, possibly an aftermath of the cessation of quinine suppression, and 13 per cent (69/532) were fresh infections, which must have been locally acquired.

Around this camp we found at various times small numbers of *A. maculatus* larvae in cleared ravine streams and seepages.

Unvisited Camps in this Region.—It was reported that Camp 6 (Changaraya) was not unduly malarious, but that the Konkoita camps to the south of Camp 1 were heavily infected. The malaria at the Tanbaya hospital camp in Burma 45 miles away was said to have been very prevalent and of severer type than that experienced in Siam. We have no knowledge of the anophelines of any of these places.

Kanburi Hospital.—The new railway had been completed by November and at the end of that month the surviving members of "F" force were withdrawn from the camps described and sent south by rail to Kanburi, a fair sized town in the cultivated lowlands of Siam, some 60 miles west of Bangkok. In the hospital here in 1 week of December there were still 12 per cent. of fresh infections (24/193), many of them probably contracted on the journey from the north. It is estimated that by this time, 8 months after leaving Changi, at least 80 per cent. of the force had had malaria.

No opportunity was given to make larval surveys around this camp, so nothing is known of the anopheline fauna

After working in this hospital for a week, we were included in a total of 3,122 men who were sent back to Changi by rail or sea in mid-December, 1943

PARASITE SPECIES (TABLES I AND III)

Plasmodium vivax was the commonest parasite Even in the early epidemic days at Camp 1, the ratio of vivax to falciparum infections was 2 1 There was considerable camp-to-camp variation in this ratio, but most of the figures for fresh infections are too small to carry much significance In view of the recognized greater tendency of vivax to relapse, it is not surprising to find the vivax falciparum ratio for all "secondary" attacks was almost 6 1, with several ratios of 13 1 or more in the later months when relapses would be commoner

All the mixed infections were mixtures of *P vivax* and *P falciparum*, *P malariae* was recognized on three occasions only

TREATMENT

(*a*) *Curative*

Quinine was supplied by the Japanese, mainly quinine sulphate in sugar-coated tablets of gramme 0 222, manufactured in Java On a few occasions dosage had to be cut down owing to shortage, but for most of the time enough was available to allow a 10-day treatment at gramme 2 a day (9 tablets) It was usual to find a good response to oral treatment, even in heavy falciparum infections, although a delayed clinical response was reported from Tanbaya hospital The interval between attacks was often very short a matter of weeks or even days It was found later in Changi that 60 per cent of the quinine-treated cases developed another attack within 10 to 15 days after the cessation of treatment.

Plasmoquine was supplied from July onwards and was used in conjunction with quinine

Atebrin was very scarce, being limited to the small amount which had been brought from Changi prisoner of war stocks, it was reserved for the few cases of quinine idiosyncrasy, and for suppressive treatment

(*b*) *Suppressive*

There was never sufficient quinine to carry out really effective suppressive treatment Attempts at suppression were nullified either by reason of inadequate dosage and/or too short a period of administration, or from the inherent deficiency of the drug as a suppressive

Atebrin was used as a suppressive drug for selected personnel, such as interpreters and medical and headquarters staff, in doses of gramme 0 2 taken twice weekly This dosage had been found to be very effective for suppressing malaria in Asian labourers in Malaya several years before the war (FIELD, *et al*, 1937), it also proved quite effective for these Europeans in Siam, for only a few developed malaria while taking it regularly, which in the light of later knowledge is perhaps surprising

DEATHS

The crude death-rate from all causes was 441 per thousand per annum, of the 6,998 men who left Changi in April, 1943, 3,087 died during the ensuing 12 months The main causes of death, acting usually in combination, were

malnutrition, beriberi, dysentery and diarrhoea, cholera, malaria, and tropical ulcers.

Malaria was recorded as the sole cause in 4 per cent. of the total deaths, and as one of the two main causes in a further 7 per cent. But several medical officers expressed the opinion that these figures, which are relatively insignificant in comparison with the death roll from other diseases, tend to minimize unduly the importance of malaria as a contributory cause of death. Loss of appetite and rapid loss of weight were the common accompaniments of attacks of malaria only too often were they also associated with a recurrence of dysentery in a convalescent patient, the reappearance of cardiac signs or oedema in an improving case of beriberi, or the rapid deterioration of a slowly healing tropical ulcer Even if the patient survived the actual attack, the ground thus lost might never be regained in this way malaria was an important contributory factor in many deaths finally attributed to some other disease.

NOTES ON THE ANOPHELINES.

No adult anophelines were caught or seen torches were not allowed and there was no other light than that of fires. As frequently happens where culicine mosquitoes are scarce hardly anyone was aware of being bitten in the huts at night, or saw any anophelines, despite the prevalence of malaria. Our findings regarding the anopheline fauna are therefore based entirely on larval surveys. In 1941 J A R. had compiled a key for the identification of the anopheline larvae and adults likely to be found in the monsoon countries north of Malaya larvae were identified under the microscope with the aid of this key Some adults were bred out to check the larval identifications.

From Table II it will be seen that the species of *Anopheles* found were *A. aitkeni A barbumbrosus A kochi*, *A leucosphyrus*, *A maculatus A minimus A vagus*

Three of these species, *A maculatus A. minimus* and *A leucosphyrus*, are recognized vectors of malaria. Just what part each played in transmission in this instance cannot be known but the parasitological evidence suggests that Camps 2 and 3 were the most malarious, and these were the only two where *A minimus* was found and *A maculatus* was abundant. *A leucosphyrus* which was present at all camps, may also have taken part in transmission it was regarded by the Allied Forces as an important vector of malaria in Burma during the wet season but the exact form of the species which we encountered could not be determined at the time nor were we able to preserve specimens. The breeding places were muddy pools, elephant footprints and the like, in or near jungle. This rather suggests the type form which appears to be the common form of the species north of Malaya and the most likely vector form, but one cannot say more than this. For further information on this subject see Reid (1949).

Anopheles aitkeni and *A barbumbrosus* do not appear to have been recorded

TABLE II

RESULTS OF LARVAL SURVEYS AROUND THE CAMPS OF "F" FORCE SIAM—1943

Date	Camp	Species													
		A aitkeni		*A barbumbrosus*		*A kochi*		*A leucosphyrus*		*A maculatus*		*A minimus*		*A vagus*	
		BP	L	BP	L	BP	L	BP	L	BP	L	BP	L	BP	L
10 June to 13 July	No 2	5	12	6	32	4	11	6	28	10	48	4	17	5	23
20 to 21 July	No 3	—	—	1	2	3	3	1	1	6	24	2	5	4	10
24 to 26 July	No 4	—	—	2	5	1	2	9	45	8	22	—	—	1	2
3 Aug to 7 Oct	No 5	—	—	—	—	2	2	9	31	3	8	—	—	2	5
Totals		5	12	9	39	10	18	25	105	27	102	6	22	12	40

BP = Number of breeding places L = Number of larvae

from Siam before *A aitkeni* was found in one area only, breeding in small pools in a water course under tall jungle with little undergrowth The larvae of *A barbumbrosus* appeared to be intermediate between the typical form of this species and *A barbirostris*, they were found several times in still water in jungle, usually under heavy shade

The breeding places of the other species were typical and call for no comment

CONTROL

On several occasions we submitted suggestions for dealing with the breeding places of vector species, but nothing was ever done owing to the reluctance of the Japanese to spare enough fit men and tools to do drainage work On one occasion, the breeding places near Camp 2 were oiled with 2 gallons of waste sump oil from the motor transport

A few large Japanese army mosquito nets were issued, under which about six men could sleep, but the need for frequent and hasty trips to the latrine at all hours of the night owing to the high incidence of diarrhoea and polyuria, made the proper use of even these few nets extremely difficult

The occasional irregular and inadequate attempts at suppression with quinine, and the suppressive atebrin taken by the very few key personnel, were the only checks so far as is known to the natural unfettered transmission of malaria in these camps

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Discussion

Comments on Malaria.—The figures for separate camps are small, as is shown in Table I and we feel that grouping them by periods will not unduly distort the facts. In addition to grouping in this way we have taken the essential step of calculating all attack rates on a common basis of infections per thousand men per day the results of this are shown in Table III and Figs. 1 and 2. These illustrate the change in incidence from fresh infection to secondary attack, and the steady rise in the proportion of vivax malaria, culminating in its almost complete predominance after the return to Changi.

Table III.

Summary of Fresh Infections, Secondary Attacks, and Attack Rates per Thousand Men per Day

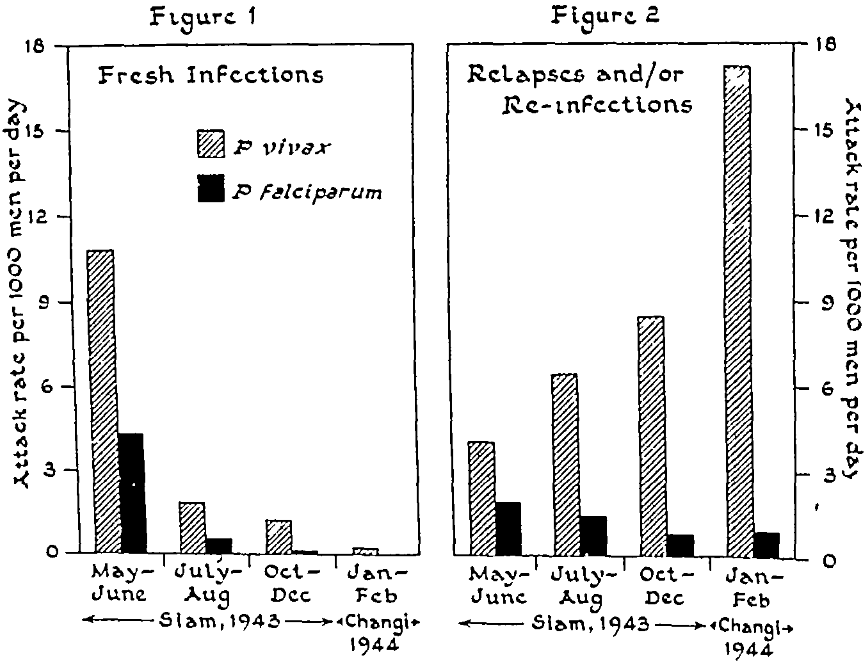
Camps.	Months.	Total man-days.	Fresh infections.			Secondary attacks.		
			P vivax.	*P falciparum.*	Total.	*P vivax.*	*P falciparum.*	Total.
1 2	May, June 1943	4 650	267 (10.8)	107 (4.3)	421 (17.1)	100 (4.1)	48 (1.9)	164 (6.7)
2, 3 4 5	July, Aug 1943	52 430	82 (1.6)	27 (0.5)	131 (2.5)	341 (6.5)	74 (1.4)	449 (8.5)
5 Kanburi	Oct.-Dec., 1943	66 490	81 (1.2)	8 (0.1)	93 (1.4)	588 (8.8)	50 (0.75)	633 (9.5)
Changi	Jan. Feb., 1944	156 100	35 (0.2)	0 —	37 (0.2)	2,690 (17.2)	137 (0.9)	2,907 (18.6)

Figures in brackets ( ) are rates per thousand men per day.
The "Total" column includes *P. malariae* and spp. unidentified.

We realise the defects of these figures. It is clear that the proportion of the force under our observation at any one time varied considerably from about one-tenth at the beginning to over three-quarters at the end, and sampling errors would vary correspondingly. A more serious defect is the one inherent in any classification of fresh infection. It is impossible to obtain a reasonable indication of the amount of transmission unless one can base the fresh infection rate not on total population as we have had to do but on the rapidly dwindling fraction of it still able to comply with our standard of freedom from fever for the preceding 12 months. Lack of information prevents us from trying to split the secondary attack total into relapses and reinfections, so far as the camps in Siam are concerned.

Back at Changi, however there was an alternative method of measuring

transmission The Japanese had started to construct the Changi airfield, a major project which involved extensive clearing of mangrove swamps and coastal coco-nut plantations, and caused disruption of drainage over a wide area The resultant increase of breeding of *A sundaicus* around the prisoner-of-war camp unfortunately coincided with the return of the heavily infected men of "F" force This combination of vector mosquito and parasite reservoir was responsible for an outbreak of malaria among the 5,000 men who had remained in Changi, producing 190 fresh cases in 7 weeks during January to February, 1944



Allowing a reasonable margin of error, this index of local transmission suggests that fresh infections and reinfections combined cannot be blamed for more than about 200 out of the total of 2,942 attacks which developed among the 3,122 members of "F" force during the same 7 weeks, or 7 per cent at most

One must conclude that the picture presented at this time was one of chronic relapsing vivax malaria, the presence of other diseases and the poor general physique of the victims perhaps accounting for the marked persistence of the infection, and the sometimes very brief interval between successive attacks

Comments on the Anophelines —ANIGSTEIN (1932), whose paper is the most

these judgements, however are based on larval surveys only and lack the definite proof afforded by trapping and dissection of adult mosquitoes.

8. Larvae of *Anopheles aitkeni* and *A. barbumbrosus* were found. These species do not appear to have been reported from Siam before.

9. We confirm ANIGSTEIN'S opinion of the malarious nature of hilly regions in Siam, but differ from him by suggesting that the season of greatest transmission in this particular region is more likely to be the wet season than the dry. He thought that agriculture in the hilly regions, which involved irrigation, usually made the malaria worse. This is in line with our findings that the two camps situated near abandoned rice fields were the most malarious.

REFERENCES

ANIGSTEIN, L. (1932). *Quart. Bull. Hlth. Org. L. N.* 1, 233.
BARRAUD, P. J. & CHRISTOPHERS, S. R. (1931). *Rec. mal. Survey Ind.* 2, 269.
CAUSEY, O. R. (1937). *Amer. J. Hyg.* 25, 400.
——— (1937). *J. Parasit.* 23, 643.
FIELD, J. W. (1941). *Trans. R. Soc. trop. Med. Hyg.* 35, 35.
——— NIVEN, J. C., & HODGKIN, E. P. (1937). *Bull. Hlth. Org. L. N.* [illegible] 236.
REID, J. A. (1949). *Proc. R. ent. Soc. Lond.* (B) In the press.
——— & WILSON, T. (1947). *J. R. Army med. Cps.*, [illegible] 149.

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STUDIES ON PROTOZOA

PART I

THE METABOLISM OF LEISHMAN-DONOVAN BODIES AND FLAGELLATES OF *LEISHMANIA DONOVANI* *

BY

J D FULTON
L P JOYNER †

National Institute for Medical Research, London

Studies of the metabolism of the *Leishmania* have been restricted, with one exception, to the leptomonad forms occurring in cultures SALLE and SCHMIDT (1928) have shown that the growth of flagellates of *L tropica* was accompanied by the utilization of glucose, and the appearance of ammonia and steam-volatile acids They suggested that there was a relationship between glycolysis and protein metabolism Several investigations of carbohydrate metabolism have been made by adding various sugars to media containing serum, in which glucose was presumably present. (KLIGLER, 1925 , NOGUCHI 1926 , SENEKJIE, 1939 , SENEKJIE and ZEBOUNI, 1940)

Both flagellates and Leishman-Donovan bodies have been shown to consume oxygen Carbon dioxide was produced in the presence of bicarbonate-Ringer solution, but was not shown to be a direct product of metabolism (ADLER and ASHBEL, 1934 , 1940) During the course of these experiments, CHANG and NEGHERBON (1947) and CHANG (1948) have reported an extensive study of three species of *Leishmania* and *Trypanosoma cruzi* in which the behaviour in culture was observed and respiration measured It was shown that while increases in the incubation temperature accelerated the growth of *L tropica*, *L braziliensis* and *T cruzi* within the range 17° to 32° C , the growth of *L donovani* showed a negative correlation with temperature increase Measurements of the pH changes in cultures showed an initial fall followed by a marked rise Appreciable amounts of succinic, pyruvic and lactic acids together with a small quantity of formic acid and carbon dioxide were identified as products of metabolism, but there is no indication that suitable control cultures were used In the presence of traces of culture medium it was concluded that glucose and fructose were oxidatively utilized while maltose and lactose failed to support respiration

* Acknowledgements are made to the following workers in this Institute Dr W J ELFORD, for determining pH values, and Mr F V WELCH and Mr C D SUTTON, for photographs The Geigy Colour Company kindly supplied the Septopaline C

† Medical Research Council Student

The present investigation was carried out with a view to determining differences in the *in vitro* metabolism of the two stages of *L. donovani*. Oxygen uptakes have been determined under given conditions and the dependence of this function upon added carbohydrate assessed. Evolution of carbon dioxide has been measured and respiratory quotients calculated. In the case of the flagellates which are more dependent upon carbohydrate substrates than the Leishman-Donovan bodies, it has been possible to examine a number of substances for their ability to support respiration. The effect upon both forms of the parasite of various concentrations of cyanide, azide and iodoacetate, which are well recognized as respiratory inhibitors, has been examined. In these experiments on inhibition were included representatives of the two main groups of chemotherapeutic agents, used in the treatment of kala-azar—the aromatic diamidines and organic antimonial compounds.

Materials and Methods.

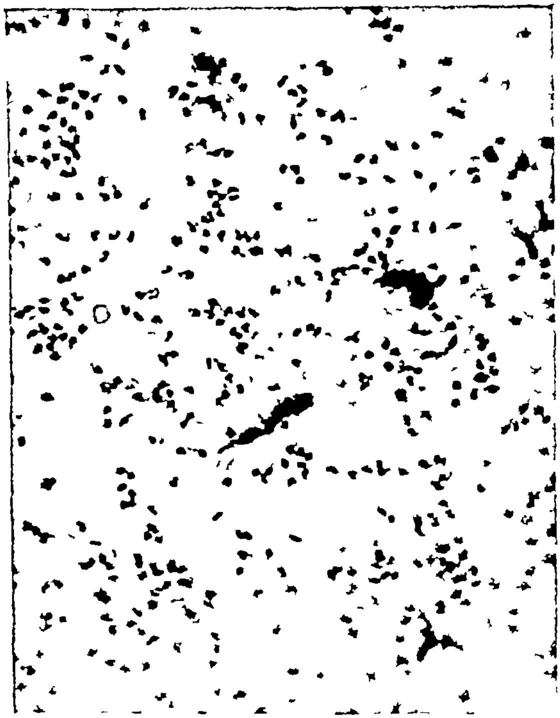
The strain of *L. donovani* used was originally isolated in 1939 from an Indian seaman suffering from kala azar and has since been maintained in golden hamsters by serial passage of infected spleen emulsions. Cultures of flagellates were from time to time derived from infected spleen tissue and maintained by subculture at intervals of 6 to 8 days in 3 to 4 ml. amounts of a liquid medium in test tubes. No strain was maintained by continuous culture in this way for more than 25 subcultures. At the end of this period such flagellates were found to be infective to hamsters. Large numbers of flagellates for each metabolism experiment were cultured in 36 ml. volumes of freshly prepared medium in six to eight 100 ml. pyrex conical flasks plugged with cotton wool. The inocula consisted of 0 25 to 0 5 million parasites derived from 6- to 8-day test tube cultures. During incubation excessive evaporation was prevented by placing a dish of water in the incubator.

The medium consisted of 30 ml. of sterile solution prepared by mixing 1 000 ml. 0·9 per cent. NaCl, 20 ml. 1 15 per cent. KCl, 2 g. glucose and autoclaving, to which were added 6 ml. of the following mixture 100 ml. rabbit serum, 60 ml. ox liver extract, obtained by steaming 1 lb. minced liver in 1 litre of slightly acid tap water for 2 hours and filtering, 2 g. Bacto peptone (Difco) dissolved in 10 ml. distilled water 40 ml. haemoglobin solution prepared by adding two volumes of distilled water to one volume of defibrinated rabbit blood and centrifuging. Before passing through Seitz filter the pH was adjusted using bromthymol blue indicator and the final value was found by glass electrode to lie between 8·0 and 8 2.

Concentration of the flagellates was effected by centrifuging the cultures for 15 minutes at 2,600 r.p.m. (diameter of centrifuge head, 54 cmm.), and resuspending the organisms in the required volume of fluid by gently agitating with capillary pipette. So far as could be judged by their motility and microscopical appearance this procedure did not damage the flagellates. They were also found to be infective for hamsters after respiration experiments. The parasite content of suspensions was estimated in a haemocytometer (Thoma) after suitable dilution and the whole ruled area of 1 sq. mm. was examined using the ⅙ inch objective and a 10x eyepiece. The mean of three counts which generally lay between 30 and 100 flagellates, was calculated.

For the separation of Leishman-Donovan bodies the extraction procedure briefly outlined by Adler and Ashbel (1940) was broadly followed. Dense plasma-albumin

PLATE I.—Discarded deposit



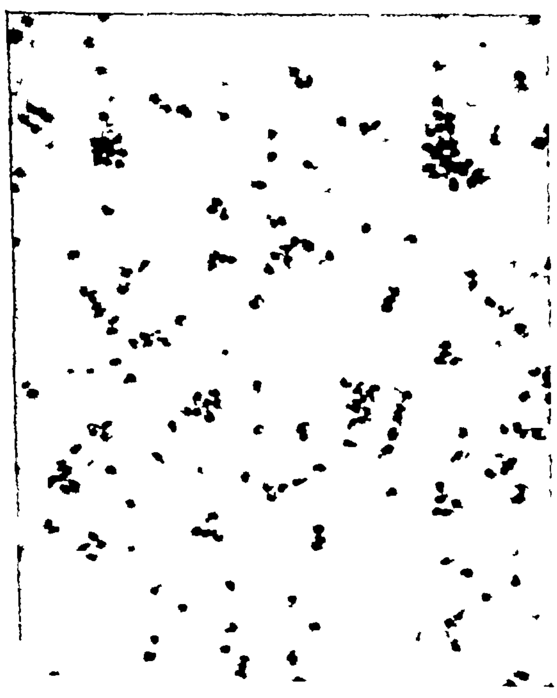


PLATE II.—Final preparation × 800

To face page 274

[illegible] (19[illegible]) to secure the differential centrifugation [illegible] ration of Leishman-Donovan bodies [illegible] the whole spleen [illegible] per cent rabbit [illegible] spleen were used for each experiment. [illegible] were drained of blood by cardiac puncture, the spleen [illegible] pounded against the sides of a mortar [illegible] 1940) containing 0·2 per cent [illegible] for each spleen used. The parasites were [illegible] and rarely more than 3 hours elapsed between [illegible] the parasites in the experimental vessels.

The [illegible] contained extremely scanty erythrocytes but no other [illegible] cells apart from Leishman-Donovan bodies, and their purity may be judged from Plates I and II. The parasites stained normally and, in an experiment in which 40 hamsters were inoculated with material previously used for respiratory measurements, all the animals became infected to the same degree as other hamsters which received at the same time equal numbers of Leishman-Donovan bodies from a fresh tissue emulsion. The Leishman-Donovan bodies were counted by mixing the suspensions in equal volumes with a standard suspension of fowl erythrocytes (CHRISTOPHERS *et al.*, 1936). The mixture was evenly spread on clean glass slides, and after staining, the proportion of parasites to at least 300 erythrocytes was obtained from two or three slides. This proved to be a satisfactory procedure, as shown by making ten separate counts by this method on one parasite suspension; the mean ratio of Leishman-Donovan bodies: red cells was 2·08, the standard deviation was 0·1 and the range 1·93 to 2·21.

For the measurement of respiration a conventional Barcroft apparatus was used according to the general technical details outlined by DIXON (1943). For the control of the temperature at 25° C. a thermoregulator with a specially large bulb containing ether and air was used. The manometer flasks had an approximate capacity of 10 ml. and 3 ml. of fluid was placed in each. Respiratory quotients were measured by the direct method of Warburg (DIXON, 1943), following the details given by CHRISTOPHERS and FULTON (1938).

Glucose concentrations were estimated by the method of MILLER and VAN SLYKE (1936), and 0·2 ml. samples were used instead of the usual 0·1 ml. as small differences were encountered. The samples of sugars used as substrates were of the "Analytical Reagent" grade of purity. Sodium pyruvate and sodium lactate were prepared by neutralizing freshly distilled samples of the acids. Sodium succinate was twice recrystallized from water before use.

For the inhibition experiments, potassium cyanide, sodium azide, sodium iodoacetate, the isethionate salts of the amidines, sodium stibogluconate and anthiomaline were commercial preparations.

RESULTS

FLAGELLATES

Changes Taking Place in Cultures

It was found by experiment that the strain of *Leishmania* used would

FIG. 1.—THE SEPARATION OF LEISHMANIA DONOVANI BODIES.

SPLEEN TISSUE
Emulsified in Phosphate-Saline (3 ml./spleen)
2,000 r.p.m.
5 min.

Deposit
+ 20 ml. P. saline
2,000 r.p.m.
3 min.

Deposit
+ 15 ml. P. saline
1,500 r.p.m.
3 min.

Deposit
+ 10 ml. P. saline
1,500 r.p.m.
3 min.

Deposit
Discarded

Supernatant Fluid (1)
+
Supernatant Fluid (2)
+
Supernatant Fluid (3)
+
Supernatant Fluid (4)
3,000 r.p.m.
15 min.

Deposit
+ 10 ml. 50% rabbit serum-saline
1,000 r.p.m.
5 min.

Supernatant Fluid
Discarded

Deposit
Discarded
(see Plate II)

Supernatant Fluid
and 4 vols. P. saline
2,500 r.p.m.
10 min.

Deposit
& 50 ml. P. saline
2,500 r.p.m.
15 min.

Supernatant Fluid
Discarded

Deposit
= LEISHMANIA DONOVANI BODIES

Supernatant Fluid
Discarded

Note.—The centrifuge used was the usual laboratory bench pattern (B.T.L. ZDC 1070).

multiply at temperatures up to 32° C, but most satisfactory results were obtained at 25° C, as judged by the numbers of flagellates present and regularity of growth, subsequent observations were made at this temperature The growth curve shown in Fig 2 was constructed by making counts of the number of flagellates present in at least 12 separate cultures at the different ages It will be seen that there was an initial lag phase up to the fourth day, followed by a period of active multiplication with a peak between the 10th and 14th days, when cytolysis and degeneration began Several of the morphological types occurring at different ages, described by CHRISTOPHERS *et al* (1926), were recognized in stained films To avoid the presence of degenerate forms, cultures up to the age of 10 days only were used

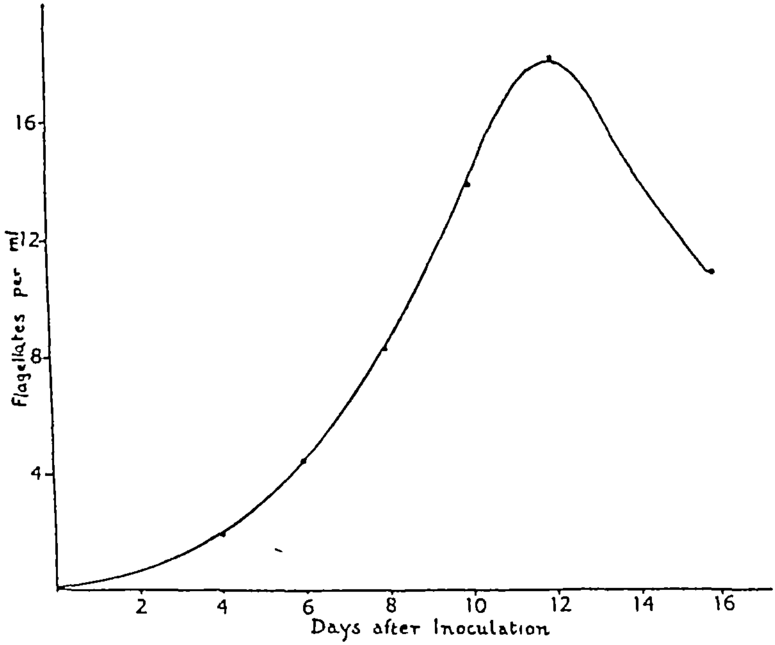


FIG 2—The growth curve of *L donovani* flagellates

Growth in the cultures was accompanied by pH changes in the medium, which were followed by measurements on samples taken from flask cultures at intervals The pH curves shown in Fig 4 for two duplicate cultures originally adjusted to different values shows an initial lag phase, corresponding to that in the growth curve, followed by a decline up to the 10th day Comparable numbers of flagellates were present in both cultures on the ninth day Repeated unsuccessful attempts were made, both before and after precipitation of protein in the medium with tungstate or colloidal iron, to detect the presence of pyruvic acid by means of the 2 4 dinitrophenyl hydrazone On the other hand, when traces of pyruvic acid were added to the medium, this substance was always

obtained and could readily be crystallized. Tests for the presence of aldehydes and alcohol were consistently negative. In order to detect the acid metabolic products, the protein from 5 litres of culture medium in which flagellates had been grown for periods up to 10 days was precipitated with the usual tungstate-sulphuric acid mixture. The acid filtrate was concentrated under reduced pressure at a temperature not exceeding 40° C. to give a final volume of approximately 100 ml. The solution was then exhaustively extracted with ether in a modified Soxhlet apparatus. The solvent was removed and yielded 0·45 g. of crystalline material which proved to be succinic acid. The non-crystalline portion from the ether, present in very small amounts, is now being investigated along with the volatile acid fraction.

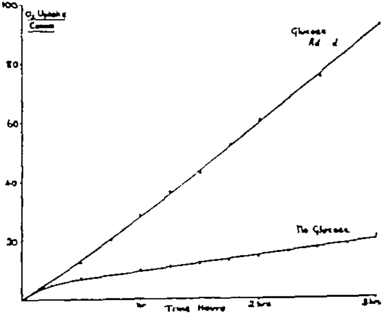


FIG. 3

The oxygen uptake of flagellates in the presence and absence of glucose

Oxygen Uptake and Glucose Utilization.

A large number of experiments were made with flagellates from different cultures, separated by centrifugation and resuspended in fresh medium. It was evident that metabolic activity varied with age of the cultures, early stages having higher metabolic rates. Details of the findings obtained from a single subculture at different ages are recorded in Table I.

Respiratory Quotients.

The values for the respiratory quotients of flagellates are shown in Table II and indicate there was no appreciable variation with age.

TABLE I

THE OXYGEN AND GLUCOSE UPTAKES OF FLAGELLATES OF THE SAME SUBCULTURE AT DIFFERENT AGES

Age of culture, days	Oxygen uptake, C.mm./hr./10^8	Glucose uptake, Mgm./hr./10^8
6	44.27	0.27
8	17.06	0.085
10	14.72	0.074

TABLE II

THE RESPIRATORY QUOTIENTS OF FLAGELLATES AT DIFFERENT AGES

Age of culture	6 days	8 days	10 days
Respiratory quotient	0.84	0.97	0.75
	0.78	0.89	0.79
		0.94	0.76
Mean	0.81	0.93	0.77

Oxidative Utilization of Various Substrates

Measurements of normal metabolic rates were made in the medium in which the flagellates were grown. Direct comparisons of the values of oxygen and glucose uptakes in the culture medium and in glucose-phosphate-saline in which the flagellates retained normal motility, showed a reduction of 20 to 30 per cent in the latter medium. In phosphate-saline free from glucose the motility of twice washed flagellates was reduced and also the oxygen uptake as shown in Fig. 3. The addition of glucose and some other sugars restored the uptake to varying extents, as indicated in Table III.

TABLE III

THE OXYGEN UPTAKE OF FLAGELLATES IN THE PRESENCE AND ABSENCE OF ADDED SUBSTRATES

Substrate	Mean oxygen uptake, c.mm. Oxygen per hr. per 10^8 flagellates	
	Present	Absent
Glucose	21.9	6.54
Fructose	15.36	3.48
Mannose	16.12	5.48
Galactose	10.44	5.62
Lactose	6.63	6.53
Sucrose	8.12	8.56
Maltose	5.14	4.43
Glycerol	8.83	8.03
Pyruvate	6.81	7.21
Lactate	5.79	5.62
Succinate	4.46	5.08
Acetate	7.97	6.23

It was shown by the Thunberg technique that flagellates were capable of reducing methylene blue *in vacuo* but this effect was not accelerated by the addition of glucose succinate or lactate. The presence of —SH groups in lysed flagellates was demonstrated by the usual nitroprusside reaction.

LEISHMAN-DONOVAN BODIES

It was found by experiment that Leishman-Donovan bodies respired equally well in glucose-phosphate saline with or without the addition of 20 per cent horse or rabbit serum as shown in Table IV when measured over a 3-hour period.

TABLE IV

THE OXYGEN UPTAKE OF LEISHMAN-DONOVAN BODIES.

Medium.	Glucose-phosphate-saline and 20 per cent horse serum.	Glucose-phosphate saline and 20 per cent. rabbit serum.	Glucose phosphate saline.
Number of suspensions	2	13	10
Number of manometric measurements	8	26	23
Mean O_2 uptake c.mm. O_2/hr./10^8 parasites	7·72	7·61	7·77
Standard deviation	0·23	1·0	1·44
Range	6·84 to 8·20	5·41 to 1 ·73	5·63 to 11·45

Since Leishman-Donovan bodies have been shown to multiply in a tissue culture medium containing rabbit serum (HAWKING, 1948), and because of its ready availability it was present in the proportion used above in subsequent respiration experiments. The values obtained for the respiratory quotients of suspensions of Leishman-Donovan bodies were respectively 0·89, 0·55, 0·59 and 0·18—no satisfactory explanation of the last figure can be given. In order to make sure that the oxygen uptake and glucose utilization noted were due to the parasites themselves, normal hamster spleens were subjected to the same separation procedure as used with infected organs. A small final deposit of structureless material was obtained which respired to a negligible extent and did not utilize glucose. Methylene blue was however reduced *in vacuo*. As a further test, suspensions containing widely different numbers were used to measure respiration, and it was found that the uptake was proportional to the numbers present. As shown in Fig. 4 the rates of oxygen uptake were approximately linear over a period of 3 hours. When previously twice washed parasites were suspended in phosphate-saline the addition of glucose gave rise to an increased oxygen uptake as shown in Fig. 5. On account of the small resulting differences, other substrates were not investigated.

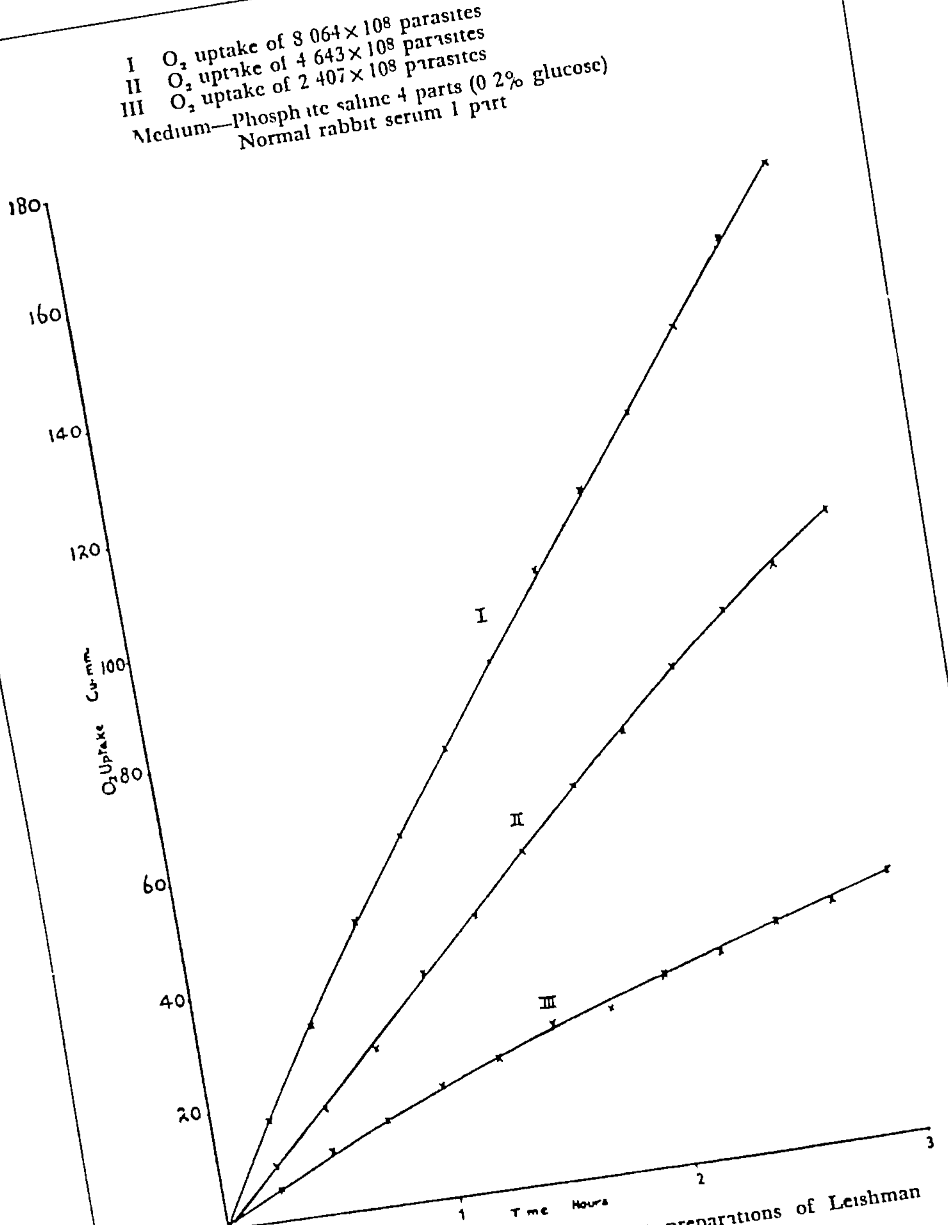


FIG 4—The oxygen uptake of three different preparations of Leishman Donovan bodies

was appreciably greater than that of stilbamidine, whereas the latter is more effective in treatment. Of the antimonial drugs, only the trivalent compound proved active both forms of the parasite were equally sensitive.

DISCUSSION

These studies have shown that the flagellate forms of *L. donovani* can be grown in a simple liquid medium at a temperature of 25° C. in numbers adequate for respiration measurements. This medium is also suitable for primary culture from infected tissues. A homogeneous medium facilitates the separation of the parasites, is satisfactory for experiments with inhibitors, and its use probably explains the difference between the form of our pH curves and those of CHANG (1948) who used a diphasic medium. Up to the present only limited studies of the nitrogen metabolism have been made but our results show that the flagellates depend to a considerable extent on glucose or other carbohydrate substrates for continued respiratory activity which proceeds at an approximately linear rate when they are present, and gradually tails off in the absence of added substrate. The consumption of glucose and oxygen approximated to that of *Plasmodium knowlesi*, but was much smaller than that of *Trypanosoma rhodesiense* (CHRISTOPHERS and FULTON 1938). Unlike these two parasites, however the flagellates appeared to be unable to utilize glycerol. The chief metabolic product was found to be succinic acid. CHANG (1948) has also noted its presence but unlike him we have been unable to demonstrate pyruvic acid in the culture medium or to show that this substance acted as a respiratory substrate.

The Leishman-Donovan bodies, which normally have an intracellular habitat, show less metabolic activity than the highly motile flagellates. They do however like the latter forms, consume measurable amounts of oxygen, evolve carbon dioxide and utilize glucose to a limited extent. Their respiration is less dependent upon the presence of added sugar than that of the flagellates, and in phosphate saline alone respiration continues, although diminished, for at least 3 hours. It appears that, as in the case of malarial parasites freed from the host cells, they are provided with some oxidizable substrate, other than glucose.

Under well-defined conditions, a comparative study of the inhibition of respiration of both forms of the parasite has been made. It is possible that the results obtained with cyanide and the amidines, by which the flagellates were affected to a greater extent, may indicate a difference in the enzyme components of the two stages. The inhibition of respiration by diamidines is in general agreement with the effects of these substances on flagellate cultures (ADLER *et al.*, 1945 COLLIER and LOURIE, 1946). But in contrast to the findings of ADLER *et al* (1948), our results show that the respiration of the Leishman

Donovan bodies was less susceptible to the action of amidines than that of the flagellates We have found that the trivalent antimonial anthiomaline at high concentrations produced considerable respiratory inhibition of both forms of the parasite whereas the pentavalent compound sodium stibogluconate was without action CHEN and GEILING (1945) compared the inhibitory effects of tri- and pentavalent antimonials on the glucose uptake of trypanosomes *in vitro*, and found the latter to be much less active In view of the differences between *in vivo* and *in vitro* findings, we consider that inhibition of respiration has definite limitations in assessing leishmanicidal activity

SUMMARY

1 A satisfactory homogeneous liquid culture medium for *L donovani* is described, and the growth curve and accompanying pH changes in it are recorded

2 The rates of oxygen and glucose uptakes of the flagellates decreased with the age of the cultures Carbon dioxide was a product of metabolism, and during multiplication the respiratory quotient remained roughly constant The parasites reduced methylene blue *in vacuo*, and the presence of —SH groups was demonstrated Succinic acid was found to be the main metabolic product

3 A method for the preparation of Leishman-Donovan bodies free from tissue, which still retained their infectivity, is described

4 Their oxygen and glucose uptakes and respiratory quotient have been measured

5 Addition of glucose to suspensions of washed parasites stimulated the oxygen uptakes of both flagellates and Leishman-Donovan bodies Fructose, mannose and galactose were found to exert a similar effect upon the flagellates

6 Cyanide, azide and iodoacetate inhibited the oxygen uptakes of both flagellates and Leishman-Donovan bodies to varying extents

7 The action of several known leishmanicidal drugs on respiration of both forms of the parasite has been investigated

REFERENCES

ADLER, S, & ASHBEL, R (1934) *Arch Zoo Ital*, 20, 521
——— & ——— (1940) *Ann trop Med Parasit* 34, 207
———, TCHERNOMORETZ, I, & BER, M (1945) *Ibid*, 39, 14
———, ——— & ——— (1948) *Ibid*, 42, 1
CHANG, S L (1948) *J infect Dis*, 82, 109
——— & NEGHERBON, W O (1947) *Ibid* 80, 172
CHEN G & GEILING, E M K (1945) *Ibid* 77, 139

Christophers, S. R., & Fulton, J. D. (1938). *Ann. Trop. Med. Parasit.* 32, 43.
—— Shortt, H. E., & Barraud, P. J. (1926). *Ind. Med. Res. Mem.*, 4, 19.
—— Sinton, J. A., & Covell, G. (1936). *How to do a malaria survey*. 3rd ed. (Revised Sinton, J. A.) Health Bulletin No. 14. Malaria Bureau, Delhi.
Collier, H. O. J., & Lourie, E. M. (1946). *Ann. trop. Med. Parasit.* 40, 88.
Dixon, M. (1943). *Manometric Methods*. Cambridge University Press.
Ferrebee, J. W., & Geiman, Q. M. (1946). *J. Infect. Dis.* 78, 175.
Hawking, Frank (1948). *Trans. R. Soc. trop. Med. Hyg.* 41, 545.
Kligler, I. J. (1925). *Ibid.*, 18, 330.
Krebs, H. A. (1935). *Biochem. J.* 29, 1620.
—— & Eggleston, L. V. (1940). *Ibid.*, 34, 442.
Miller, B. F., & van Slyke, D. D. (1936). *J. Biol. Chem.* 114, 583.
Noguchi, J. (1926). *J. exp. Med.* 44, 327.
Salle, A. J., & Schmidt, C. L. A. (1928). *J. infect. Dis.*, 43, 378.
Senekjie, H. A. (1938). *Amer. J. trop. Med.* 18, 601.
—— & Zebouni, N. (1940). *Amer. J. Hyg.* 34, 67.

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A CASE OF INDIGENOUS KALA-AZAR IN THE GAMBIA *

BY

J H WALTERS, M D , M R C P, LIEUT -COLONEL, I M S (retd)

Since kala-azar has rarely been recognized throughout the West African Colonies, publication of the following case appears justified

Musa Jobe, a boy aged 15 years, was admitted to the Medical Research Council's Field Research Station at Fajara, The Gambia, on 16th July, 1948, for investigation of a disease comprising wasting with progressive enlargement of the liver and spleen

He was born and had always lived in Gunjur, a coastal town near the southern boundary of the Protectorate, and had only left it to make occasional short visits to Bathurst, which lies at the mouth of the Gambia river, about 30 miles away His illness had begun insidiously several months before his admission, but it was stated that his spleen had been enlarged since early childhood

His parents were healthy and, together with their five other children, showed no signs of leishmaniasis

On admission, the boy was thin, his musculature poor, and the outlines of the greatly enlarged spleen and liver were visible through the lax abdominal wall The liver was palpable to four fingers' breadth below the costal margin, the spleen to six fingers' breadth , both organs were smooth, firm and painless The only other abnormalities found were a papilloma of cherry size above the pubes, which on section was found to be free from *Leishmania*, and a chronic ulcer above the left ankle

Despite an irregular low fever with an afternoon rise to 100° to 102° F , he ate well and suffered no discomfort other than a dragging sensation from the weight of the enlarged spleen

Routine investigation showed a normocytic, orthochromic anaemia of moderate

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* I am greatly indebted to the authorities listed for information concerning the incidence of leishmaniasis in their respective territories , to Professor H E SHORTT, C I E., of the London School of Hygiene and Tropical Medicine, for confirmation of the presence of *Leishmania* in the smears of splenic pulp , and to Professor B S PLATT, C M G , of the Medical Research Council, for permission to publish this report I wish also to thank Mr R PREECE, of the Human Nutrition Research Unit, who prepared the sections and the photomicrographs

degree and granulopenia. The stools contained ancylostome ova. The detailed haematological findings are tabulated later

Although no parasites could be found in thick blood films taken during febrile periods on three successive days, chronic malarial infection was considered the probable diagnosis, and specific treatment was begun with quinine hydrochloride grain 15 and plasmoquin gramme 0·01 daily iron as ferrous sulphate grain 10 daily being given in addition. Liver biopsy was performed and the tissue obtained was sent to London for section the results were not available for 3 weeks.

A review of the case 1 week later showed that no progress had been made The fever was unabated, and although the spleen had receded slightly the liver appeared to have become further enlarged.

The blood picture showed some response to iron therapy but a marked granulopenia persisted. The total serum protein was 8 gramme per 100 ml., and the serum showed a positive reaction to NAPIER's formo-gel test. Smears made from material obtained by splenic puncture showed the presence of typical Leishman Donovan bodies in very large numbers lying free in the splenic pulp.

The diagnosis of kala-azar having been established, an intensive course of pentavalent antimony was prescribed. The preparation used was neostam (B.W and Co), which was given by intramuscular injections on alternate days.

When six injections, comprising gramme 0·575 had been given an unusually severe toxic reaction occurred and the course of treatment had to be terminated. The patient developed high fever vomiting and diarrhoea, a generalized urticarial rash, and painful induration over the injection sites in the buttocks. Haemolysis was suggested by a faint trace of icterus and a fall in the red cell concentration from 4·08 to 3·01 m. per c.mm. and in the haemoglobin concentration from 10·4 to 7·0 gramme per 100 ml. An increase in the granulocyte count had, however occurred.

This acute reaction subsided after 6 days but necrosis of tissue occurred at the injection sites with the formation of large ulcers below both iliac crests.

Splenic puncture was then repeated. Smears of splenic pulp still contained Leishman-Donovan bodies, though now only in small numbers, which indicated the need for further specific treatment. It was, therefore, decided to give a course of stilbamidine isothionate (M. & B.) consisting of 10 daily intravenous injections, totalling gramme 0·675

After this treatment, rapid improvement took place the patient remained afebrile and quickly gained weight, while the liver and spleen diminished in size and the ulcers over the buttocks healed.

Spleen puncture and liver biopsy were repeated 3 weeks after the completion of the course of stilbamidine. Smears of splenic pulp were now free from *Leishmania*.

Convalescence was interrupted by an attack of subtertian malaria, after which he was discharged in good condition but with a persistent degree of splenic and hepatic enlargement, each organ remaining palpable to the breadth of four fingers below the costal margin.

The results of blood examinations made during his illness are tabulated on opposite page.

TABLE OF HAEMATOLOGICAL FINDINGS

	16 7 1948, on admission	15 8 1948, after anti-malarial treatment.	29 8 1948, after six injections of neostam	12 10 1948, after course of stilbamidine
R.B C in millions per c mm	3 43	4 08	3 01	4 14
Haemoglobin in g per 100 ml	8 5	10 4	7 0	9 7
M C V in c. microns	81	73	80	74
M C H C in per cent.	31	35	29	32
W B C per c mm	2,500	3,150	4,650	21,000
Per cent Neutrophil polymorphs	33	27	69	27
„ Eosinophil ,	2	1	0	8
„ Lymphocytes	62	57	24	60
„ Monocytes	3	15	7	5
Total serum protein in g per 100 ml		8 0	6 4	7 9
Formo-gel test		+		0

Liver biopsy was performed initially as a routine measure in an investigation into the histo-pathology of tropical hepatomegaly. It was repeated 3 months later when, despite apparent eradication of the leishmanial infection, a considerable degree of liver enlargement persisted.

Sections prepared from the first specimen showed an intense degree of parasitization of the liver in which the Kupffer cells were grossly swollen and were tightly packed with Leishman-Donovan bodies (Plate I), while some cells of the liver parenchyma itself had been invaded. Numerous parasite-laden histiocytes were present in the portal spaces, and in addition there was a moderate degree of lymphocytic infiltration of both the portal tracts and sinusoids.

A considerable degree of fibrous hyperplasia was present in the portal tracts, with thickening of the adjacent reticular fibres within the lobules, while at the periphery of the lobule, moderate fatty infiltration of the hepatic cells was also evident.

Three months later, after treatment, the Kupffer cells had shrunk to their normal size and no Leishman-Donovan bodies were visible. A dense lymphocytic infiltration persisted in the portal spaces and numerous lymphocytes were still present in the sinusoids. (Plate II.)

In the portal tracts the degree of fibrous hyperplasia was unchanged and abnormal thickening of the reticular fibres within the periphery of the lobule persisted, but fatty deposits were no longer evident.

The residual degree of hepatic enlargement was apparently due in part to the heavy cellular infiltration and in part to hyperplasia of the stroma, in view of which it seems unlikely that the organ can ever resume its normal size.

Discussion.

It is difficult to determine the extent of the disease in a zone of hyper endemic subtertian malaria, such as the Gambia, where wasting, splenomegaly and recurrent fever constitute a commonplace syndrome arousing no special interest. It is probably rare, however, since in the 5 months which have elapsed since the recognition of the case here reported, no further example has been found.

The source of disease from which this boy received his infection is not known. Three possibilities exist, namely:

(i) That the disease was transmitted from an infected dog, as in Mediterranean kala-azar.

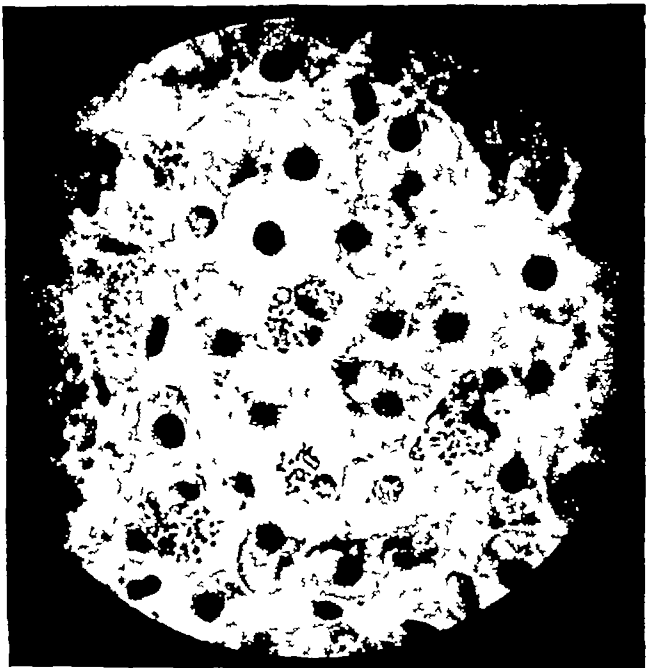
(ii) That the disease was introduced by a traveller arriving from a distant endemic area, perhaps by a soldier who had returned from the Burma campaign.

(iii) That the infection was acquired from an unrecognized endemic human case.

Leishmaniasis other than kala-azar is known to exist in territories adjacent to the Gambia. Thus, canine leishmaniasis is prevalent throughout Senegal while human dermal leishmaniasis has been observed in the Senegal, the French Niger Province, and in Dahomey*. It may well be, therefore, that these conditions exist also in the Gambia.

* Médecin Général C. Durieux, Dakar. Personal communication.

PLATE I.—Section of liver biopsy specimen taken before treatment, showing Kupffer cells heavily parasitized. A liver cell at the top of the plate also contains Leishman Donovan bodies. × 630



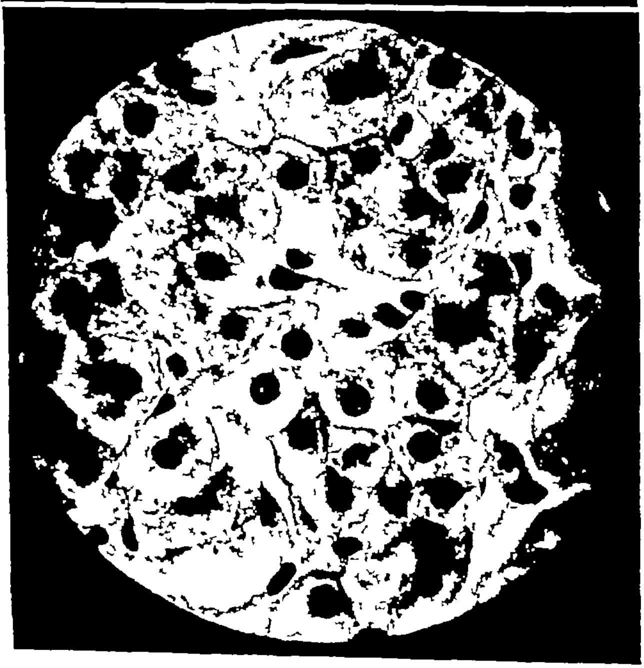


PLATE II.—Section of liver biopsy taken after completion of treatment. No parasites remain. × 630

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Although kala-azar has not been diagnosed in Sierra Leone, where a number of suspected cases have been examined by spleen puncture,* recent reports suggest that the disease is endemic throughout the northern districts of Nigeria † Reports from that colony show that the disease has been diagnosed with increasing frequency of recent years, the figures being

	Case		Cases
1936	1	1943	11
1937	1	1944	10
1939	1	1945	23
1942	1	1946	7
		1947	20

It is not known in how many instances the parasite was recognized and in how many the diagnosis was based on the clinical findings supported by a positive formo-gel test It should be remembered that the results of this test require to be interpreted with caution in tropical Africa, since a positive reaction, in every way resembling that obtained in the case of kala-azar, may be shown by the serum of a patient suffering from trypanosomiasis

Farther to the east, endemic zones are found in the Sudan, especially in Dafur Province, while military operations in the late war revealed hyperendemic foci in Ethiopia, at Gedabia and Galebat, and in Kenya at the northern end of Lake Rudolf ‡

Although the possibility that the patient here reported had received his infection from a canine source cannot be excluded, the absence of human kala-azar in adjacent French territory, where canine leishmaniasis is widespread, suggests that it is improbable Nor is it likely that he had acquired his disease from an infected traveller who had carried it from a distant endemic area, for few such visit this isolated town, and over 2 years have elapsed since the return of Service men from the Far East

However, it is not difficult to visualize an endemic zone of diminishing intensity spreading westwards from the Sudan along the ancient routes which skirt the southern fringes of the Sahara to reach the Atlantic coast

Possible sand-fly vectors certainly exist in the territories mentioned, but little is at present known of their species Among a multitude of mosquitoes and other insects captured in the over-filled and dilapidated trading store in which this patient lived, a number of sand-fly were recognized, but the specimens sent for identification were lost in transit An attempt to obtain further specimens will be made in due season

* Dr P C Cosgrove, Freetown Personal communication
† Director Medical Services, Nigeria Personal communication
‡ Paper by Brigadier Sidney Smith, Conference of Services Physicians, Cairo, 1942

SUMMARY

1 Attention is drawn to the occurrence of kala azar in the Gambia by the recognition of a case in which the diagnosis was confirmed by spleen puncture and liver biopsy

2. The disease had been acquired locally

3 Possible sources of infection are considered. It is suggested that the disease is endemic in a zone bordering the Sahara and extending from the Sudan to the West Coast.

4 Investigation into the species of possible sand-fly vectors is required.

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A SCORBUTIC DIET IN A NILE CATARACT COMMUNITY

BY

N L CORKILL *

The Batn el Hagar, " Belly of Stones," is an area south of the Second Cataract and east of the Nile between Dongola and Wadi Halfa It is rainless and the desert abuts on the river A few meagre strips along the steep banks are available for limited cultivation This varies to some extent with the level of the Nile flood or *damira*, which is roughly from June to August The inhabitants are Nubian Mahass and Arab Garrarish Medical officers speak of them as malnourished On the 28th January, 1946, the writer visited the main village, Attiri, and with limited time and means attempted a rough assessment of the local diet and the nutritional state of the people

Tobacco was the poor but main cash crop Formerly it had been of more value, for some years back it was sold freely in local markets, and it was said that a considerable amount was smuggled into Egypt In 1946 most of it was taken by camel to Halfa—grain, sugar, tea, chillies, hibiscus, etc , being brought on the return There was considerable domestic use of tobacco Apart from being smoked in home-rolled cigarettes, it was chewed by most people The only other cash crop appeared to be a very small quantity of lupins, *Lupinus termis*, called *termus*, exported to Egypt

FOODSTUFFS AND THEIR UTILIZATION

The foodstuffs used were few Meat was rarely eaten and fish appeared to be eaten only in the flood season The milk animals were few and their yields were said to be small compared with those in other parts of the country, which might be expected in view of the very little forage available Table I

* The writer acknowledges with pleasure his indebtedness to Dr A J HENRY, Government Chemist, Khartoum, for the analysis of the sample of natron

shows the yield by seasons as estimated by a group of the villagers themselves. Family A, for instance that of the head man, possessed two she-goats, one only of which was in milk. The amount available for the average day's diet was measured as 7 oz. In family B, the only milk was a little brought from the neighbours—family A. Family F possessed a she-goat and a ewe, but neither was in milk. Ewes did not appear to be expected to yield any milk for human consumption at all. Family G were getting 2 pints from a she goat and none from a ewe, a cow and a she-camel. Usually no butter was made.

The staple was millet, *Sorghum caudatum*, of the white variety known as *feterita*. Wheat was used by the household of the headman only Small gourds (probably *Cucumis* sp.) called *jurma* were eaten by some. Bulb-onions, chillies,

TABLE I.

[illegible] DAILY YIELDS OF MILK IN *Rotls* (ROUGHLY PINTS) AVAILABLE FOR HUMAN CONSUMPTION IN DIFFERENT SEASONS, AS ESTIMATED BY THE VILLAGERS.

Animal.	Winter	Summer	Flood.
Cow		1	0
Camel	2	½	0
Goat	1	¼	0
Sheep	0	0	0

edible hibiscus, *H. esculentus* called *weika* salt, tea and sugar figured in most diets. So—for 4 months a year—did the leaves of a bean, apparently *Dolichos lablab*. The beans themselves, it seemed, were only eaten for 2 months, June and July Family C said they would use dried peas, *Pisum sativum* if they were not so dear family H, however used them.

Dates were so little available as to be negligible though a fermented date wine *dakai*, was occasionally obtained and drunk in the hot season Sesame oil was beyond the means of most houses and all households stated that any available was used by the women as a skin application. Family G said they had perhaps a pint (*rotl*), of sesame oil in a month, and it was used for this purpose.

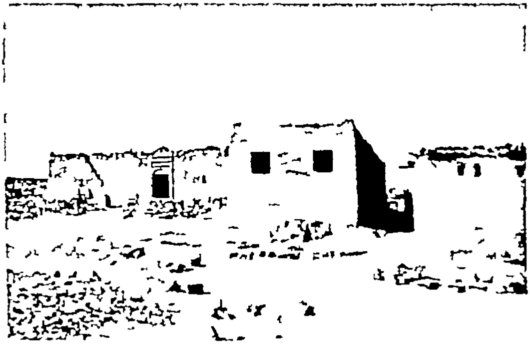
An interesting point about the dried hibiscus was noted. As drawn from a sack, tin or box, the last portions to be taken out contain most of the seeds which have fallen to the bottom. Presumably it is these seeds which contain the important nutrients, and it would be interesting to know if there is any folk-appreciation of this point, *e.g.*, whether merchants charge more for the tail-end contents of a sack.

The grain was coarsely ground in the rotary quern called *rahaya* and any finer grinding was done on the rubbing grindstone called *murhaka*. Leavened

The Batn el Hagar. It is arid and rainless. The embankment is a relic of Kitchener's railway

Attiri village—seen from the east bank of the Nile looking across cataracts

On the east bank of the Nile opposite Attiri. Camels of the local Gararish setting out for Halfa with loads of tobacco



Attiri village. Typical mud house

Attiri village. Landing place and steep banks with scanty crops of tobacco and lupins mostly exported, and beans (*Dolichos lablab*) the leaves of which provide carotenoid, ascorbin, riboflavin and calcium

Attiri village. Grinding millet in the rotary quern called *rhaiya*. In the foreground is seen a rubbing grindstone called *murhaka* and used for finer grinding

wheat loaf, *gorasa khamira*, was eaten, usually, only by the household of the headman Most of the community ate a millet porridge, *asida*, and the fermented wafer-like *kisra* or *fetir* made by cooking a paste of millet-flour spread thinly on a hot iron plate All cereal foods were eaten with relish, *mulah*, of which there were three main kinds For 4 days a week, fresh bean leaves were cooked with onions, dried hibiscus, chillies, and salt, this relish was called *mulah warag el lubia* or *mulah khadra*, the same without the bean leaves was used 2 days a week and was called *mulah weika*, and once a week the aim was to use the third relish which contained the gourd, chillies and salt only, this was called *mulah jurma* Cooking of the relish was done from the cold state and lasted at least an hour, that for family D was timed, and took 75 minutes

Tea was commonly drunk on rising, say at 6 to 7 a m, and also about 4 p m in the afternoon Any milk available appeared to be used in the tea Meals proper were normally two a day, either breakfast, *fatur*, eaten early in the morning, or lunch, *ghada*, eaten at noon, and always supper, *asha*, just before dusk Family C said they fed their dogs from the common table, *i e*, the dog's food would come from the foodstuffs taken out and weighed as typical of the day's food Normally, at breakfast or lunch, millet porridge was eaten, and at supper, either the wheat loaf (headman's house only) or the millet chupatti Whichever cereal food was eaten was accompanied by a relish

The foregoing was said to be the characteristic diet for some 4 or 5 months of the year During the river flood it was said that the beans were eaten, also a little fish, and also a little of the date wine A local weed collected and eaten also at this time as a herb in the relish was spoken of as *girgir*, a word used elsewhere in the Sudan of the cultivated *Eruca sativa*, the garden rocket Presumably, it is as the river drops that the cultivation is mostly done, producing the bean leaves until the hot dry season about mid-year Some of the figures recorded appeared to be incompatible with the official ration issues being made at the time, but no doubt barter was going on between families, *e g*, grain, sugar and bean leaves for milk

In addition to its economic aspect, tobacco appeared to play a direct role in the nutrition of the community, for young and old of both sexes chewed it, and with it natron, *atrun*, especially the type brought from Dongola and called *atrun binni* In family A, an adult man chewed tobacco seven times daily, and his son of 11 chewed also In family B, half a *ruba* (near 250 g) a month was said to be chewed by the father and a similar quantity by the son Most women chewed, though not all

Table II shows the composition of this natron It was said that 20 oz (*wagiya*) of tobacco for chewing would be mixed with eight of the natron, the product being called *saffa* A " chew " of this would be retained in the mouth for about half an hour and then spat out If this were done four times a day—probably an underestimate for most users—the amount of sodium,

chlorine and iron taken in during the day from this source alone was probably quite high, though an estimate is difficult to arrive at. Natron, as well as common salt, was added to the relish for cooking, and these thus furnished still more minerals to the intake.

NUTRITIONAL VALUE OF THE DIET

With the co-operation of the head of the community eight houses were visited and the day's food intake estimated by requesting the housekeeper —generally a wife—to set out on the floor the quantities of foodstuffs to be used in preparing the day's food. Food already eaten or in course of preparation was allowed for by bringing fresh supplies either from the family store or from a neighbour Milk already drunk was demonstrated and measured as water The foods were then weighed The sex and age of the persons feeding in the house were noted and, later reduced to what was considered to be a sufficiently reasonable approximation to man values to give a practical idea of the community Table III shows the weight values obtained, and Table IV the nutritional values as worked out from an average of the seven diets remaining after disregarding that of the head of the community whose feeding was anomalous in that on the one hand he was a comparatively wealthy man, and on the other had unusual obligations of hospitality

Table III calls for little comment. The pattern of feeding described above, and derived from preliminary questioning was on the whole borne out by the food found and weighed in the eight homes.

Table IV shows that the bulk of the calories came from millet. The protein is shown to be above the 100 g level and to be derived mainly from millet indeed, this cereal supplied most important dietary quantities except for useful contributions from the bean leaves, of calcium, carotenoids, riboflavin and ascorbin. In the absence of local assays, the values for bean leaves have been assumed to be sufficiently well represented by those given by PLATT (1945) for fresh dark green leaves. These may of course be wide of the mark.

The diet is contrasted in Table IV with what the writer considers a reasonable working standard in warm climates for a 70-kg man when the daily mean temperature is below 85° F effective temperature. No heavily built persons were seen and the average body weight was certainly less than 70 kg At the time the daily mean temperature was perhaps about 70° F with a strong wind blowing for most of the time. The calories call for no comment. Animal protein is lacking and fat is very short. Calcium from the diet is on the low side, but no doubt the natron used in the relish and that present in chewed tobacco would bring up the intake considerably Iron seems safe, carotenol through the provitamin seems safe also and thiamin and niacin are plentiful. There is some 30 per cent. deficiency in the riboflavin. As all items contributing ascorbin to the diet were cooked from the cold state with soda for upwards of an hour it would seem improbable that much or even any ascorbin would remain undestroyed. Unfortunately no chemical tests could be done

TABLE II

ATTIRI PERCENTAGE COMPOSITION OF THE NATRON, *Atrun bmm*, USED FOR (A) MIXING WITH CHEWING TOBACCO AND (B) FOR COOKING OF VEGETABLES.

Sodium carbonate	30 63	Magnesium carbonate	0 13
Sodium bicarbonate	7 81	Iron oxide	0 45
Sodium chloride	6 61	Potassium	A little, not estimated
Sodium sulphate	25 40	Water of crystallization	4 99
Calcium carbonate	0 70	Acid insoluble material (sand, etc)	22 83
		Organic material, probably humus	A little

TABLE III

ATTIRI DIET OF EIGHT FAMILIES, ANALYSED BY FOODSTUFFS, AND REDUCED TO DAILY MAN-VALUES IN GRAMMES *

Family	Milk.	Eggs	Butter	Peas	Millet	Bean leaves	Gourds	Bulb onions	Chillie peppers	Dried hibiscus	Cane sugar	Tea	Salt.	Natron
A†	2 3	0 33	0	0	2,666‡	250	111	10	2	8	35	7	33	6
B	2 8	0 17	0	0	482	178	0	14	2	2	52	10	39	2
C	0 2	0 00	0	0	833	166	100	9	1	2	78	6	25	3
D	2 3	0 00	0	0	615	86	0	0	0	1	30	3	15	3
E	0 8	0 00	0	0	642	107	0	5	0	7	28	1 3	19	2
F	0 7	0 00	0	0	687	143	0	53	1	10	32	1 7	15	6
G	2 7	0 27	0	0	1,055	222	0	87	1	24	42	2 0	27	5
H	2 4	0 00	3	74	251	112	0	85	1	32	42	2 0	30	1
Total†	11 9	0 44	3	74	4,565	1,014	100	253	6	78	304	26 0	170	22
Average†	1 7	0 06	0 4	10 5	652	145	15	35	1	11	43	3 7	25	3

* The man-values factors used were —
0–2, 0 2 2–3, 0 3 3–5, 0 4 5–7, 0 5 7–9, 0 6 9–11, 0 7 11–13, 0 8
Females, 14–50, 0 8 Those over 50, 0 8 Males, 14–50, 1 0

† That of the head of the community , it has unusual hospitality needs and is therefore excluded from the Total and the calculation for the Average

‡ Includes a large proportion of wheat

NUTRITIONAL STATE

No plump persons were encountered No rachitic residues were seen Night blindness was known of but was said to be rare A burning pain in the extremities, which is not infrequently encountered in the northern and central Sudan with some peculiarities of distribution, was not, it appeared, an important

TABLE IV

ATTRIBUTED NUTRIENT VALUES IN THE AVERAGED DAILY DIETS OF SEVEN FAMILIES (VALUES IN CAL, G AND MG)

Food items	Quan	Cal	Prot	Fat	Ca	Fe	Carot	Thiam	Rib	Nia	Asc.
Milk	1 70	1	0 1	0 0	2	0	2	0 000	0 003	0 00	0
Eggs	0 06	0	0 0	0 0	0	0	0	0 000	0 000	0 00	0
Butter	0 40	0	0 0	0 0	0	0	10	0 000	0 000	0 00	0
Millet (Sudan value)	652 00	2 500	104 0	20 0	208	44	0	3 200	0 782	23 82	0
Peas	10 50	32	2 3	0 1	6	0	21	0 017	0 183	0 29	0
Bean leaves (less 20%)	145 00	52	4 8	0 5	252	4	15,600-c	0 180	0 300	1 02	(120)
Onions (less 5%)	35 00	12	0 3	0 0	10	0	80-c	0 010	0 033	0 03	(5)
Gourds	15 00	3	0 0	0 0	2	0	0	0 005	0 006	0 08	(1)
Chillie peppers	1 00	3	0 0	0 0	2	0	130-c	0 000	0 000	0 00	(2)
Dried hibiscus	11 00	?	?	?	?	?	?	?	?	?	?
Sugar	43 00	172	0 0	0 0	0	0	0	0 000	0 000	0 00	0
Tea	3 70	1	0 3	0 0	1	0	0	0 000	0 004	2 25	0
Salt	25 00	0	0 0	0 0	0	0	0	0 000	0 000	0 00	0
Natron	13 00	0	0 0	0 0	36	44	0	0 000	0 000	0 00	0
Total	—	2 785	112 0	20 6	517	92	15,843-c	3 502	1 317	27 40	(128)*
Standard	—	3 000	100 0	50 0	800	15	1,500 (4,500-c)	1 0	2 0	22 0	50
Important deficiencies	—	-215		-30	-300				-0 683		-50

* Considered nil as cooked from the cold state with a soda for over an hour

local affliction, though it was known, and apparently called *jugi*—presumably a Mahass word Table V shows the degree to which were present certain signs, either accepted as being significant of malnutrition, or suspected by the writer (CORKILL, 1948) to be so, because of the greater degree to which he has found them present in Africans in conditions of malnutrition as compared with other Africans not so obviously malnourished Children below 12 are excluded from the series

Follicular keratosis is believed by the writer to be indicative in natural circumstances of joint carotenol and ascorbin deficiency It was present only to a slight degree. A thickening of the interpalpebral conjunctiva with a deposit of pigment is considered by the writer probably to represent a chronic form of xerophthalmia. It is common in Sudanese present to different degrees in different areas, more marked in the poor than in rich and in males than in females, increases with age, is sometimes associated with Bitot s spots, and has an association in a proportion of cases with pterygium or pinguecula. In this Attiri

TABLE V

ATTIRI ARBITRARY VALUES FOR KNOWN OR ASSUMED SIGNS OF MALNUTRITION IN CERTAIN GROUPS.
(The values were obtained by scoring for each sign, 0 if absent, 1 if just appreciable, 2 if present to moderate degree and 3 if markedly present, and then taking the average as the index value.)

Group.	Number	Foll. kerat.	Chronic xeroph.	Mosaic skin.	Dyseb.	Ging. livid.	Buccal livid.	Glossal livid.	Buccal friction.
Attiri, 1946									
( ) Non scurvy both sexes, 11 upwards*	15	0·1	0·4	0·8	0·3	1·2	1·0	0·6	1·2
(b) Scurvy both sexes, 11 upwards ...	5	0·0	0·4	0·6	0·0	1·4	0·4	0·2	1·0
Kassala, 1942									
100 cases of ambulatory† polyhypovitaminosis									
(a) Severe	12	1·0	1·6	2·5	1·5	1·5	1·3	1·0	0·8
(b) Moderately severe	23	0·8	1·4	2·3	1·2	0·7	1·0	0·9	0·8
( ) Mild	65	1·3	1·6	1·3	0·7	1·0	0·9	0·8	0·6

Excluded are members of the families not examined and those below 11 years of age.

† CORRELL *et al.* (1948.) Observations on p.o.w community in which were occurring cases of malnutrition dominated by pellagra but also with cases of scurvy wet beriberi and hyporiboflavinosis.

community its degree of incidence was, for Sudanese, in the writer s experience, quite notably low and possibly may be accounted for by the high intake of carotenoids, from the leaves of the bean and the so-called *girger*

Mosaic skin (considered a sign of niacin deficiency) was also not very marked. The dysebacia (considered a sign of riboflavin deficiency), formerly known in the literature of pellagra as "sulphur flaking, was also low—among many tribal communities in the Sudan it is strikingly common. No cheilosis was seen and no canthal glazing Gums showed a rather high degree of blueness. Tongues were pink rather than the magenta usually shown by most tribal Sudanese. There was less of the blue purple and black patching on the tongue, seen so often elsewhere in the Sudan. The buccal mucosa was, like

the gums, bluish Not one of all mouths looked at was free from caries or the gaps denoting missing teeth An impression of the teeth on the inside of the cheeks (an appearance which may be conveniently referred to as the buccal frieze), and is suspected by the writer to be possibly indicative of thiamin deficiency, was fairly well defined

In examining the inhabitants for the degree to which these signs were present, a certain number of cases of disease came to light A man from a family not included in the survey was carried to the writer In the left calf was an intra-muscular sinus discharging a thin pus There was said to have been no obvious cause, but about a year before there suddenly occurred a painful and crippling swelling in the calf which ultimately discharged Scurvy seemed a probable diagnosis His gums were not bleeding, but the examination and enquiry revealing this resulted in the immediate bustling forward of a nearby woman of 26 with bleeding gums, though no classical scurvy buds Bystanders then volunteered that swelling in the limbs and "bleeding teeth" were commonest 2 and 3 months later towards the end of the hot dry season, the *sef*, which is what might be expected in such seasonal and dietary circumstances

Five other cases which it seemed reasonable to diagnose as scurvy were seen in the same community In family A, a woman of 25 had bleeding gums In family B, a woman of 30, with what appeared to be a resolving pneumonia, had bleeding gums and scurvy buds In family C, a girl of 17, with a limp, gave a history of the appearance of a painful swelling in the left thigh about a year before, from which she had not yet fully recovered Inspection and palpation revealed nothing except that the hip joint itself appeared to be unaffected Her brother, a boy of 15, had scurvy buds In family G, a woman of 30 had scurvy buds Thus, examination of 25 persons available from the eight households revealed five cases considered tentatively at the time as scurvy, and these, with the case of abscess in the calf, gave six cases in all, seen in 1 day in one small village

In Halfa hospital, several days later, four more cases were seen which had been, or were subsequently, diagnozed as scurvy They had all come from the local area A boy from Abri had limb haematomata, a man from Debeira, and another from Halfa, had either haematomata or bleeding gums, or both (the field notes are not clear on this point), and a Nuba railway employee from a nearby station in the Bayuda Desert had haematomata of the calf and thigh muscles and scurvy buds of the classical type

Discussion and Conclusions

The diet for most of the year appeared to be deficient in ascorbin in that it was destroyed by cooking, scurvy cases were found, their occurrence with a seasonable peak, as might reasonably be expected, was described by the people, and further cases were found in the local hospital in patients deriving from the same or an adjoining and similar environment In addition, carious teeth or

gaps denoting missing teeth were found in every person examined. The dietary and clinical findings thus appear to be complementary but it would be of considerable interest (*a*) to have a more exact assessment of the nutrient values of the diet and its constituent items—more particularly the bean leaves, both before and after cooking and (*b*) a measurement of the ascorbin values of the leucocytes of a sample of the population.

The food situation in the Batn el Hegar it seems, might be bettered by a long term policy in the planting of the jujube tree, *Z spina-christi* the gingerbread nut palm, *H thebaica*, the desert date, *B aegyptiaca*, and the mesquite or honey locust, *P juliflora* which, if they will grow locally as seems probable, will provide calories, oil, calcium, carotenoids, ascorbin poultry food and animal fodder and thus indirectly animal protein, firewood, baskets, matting, and vegetable ivory No doubt these possibilities, as well as that of re-settlement, have been already considered.

Summary

(1) A Nile community living in an arid area, and its restricted food economy are described. It was reputed to be malnourished.

(2) The daily food intake was assessed by weighing an estimated day's food for each of eight families and averaging the man-day values for seven of them which appeared to be characteristic of the village.

(3) The diet was considered to be deficient in ascorbin, as the only foods contributing this were probably cooked for over an hour from the cold state and with an alkaline cooking soda.

(4) The villagers were lean, and in every mouth looked at there were caries or the gaps denoting missing teeth. An old case of scurvy was seen, active cases of scurvy were found in the village, and the people described a local seasonal peak of "bleeding teeth and swellings in the limbs. Cases of scurvy were also found in a nearby hospital in persons deriving from the same or similar local environments.

(5) It is suggested that in such circumstances resettlement is indicated, but that as palliatives, certain trees yielding useful food or economic products may be introduced.

References

Corkill, N L., Creditor, H. & Stewart G E. S (1948). *J trop Med. Hyg* 51 160

Platt B. S (1945). *Tables of Representative Foods Commonly Used in Tropical Countries* London H.M.S.O

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THE ROLE OF FAT INGESTED IN THE DIET IN THE INCIDENCE OF SPRUE *

BY

J C GILROY, COLONEL R A M C (retd)
Baragwanath Hospital, Johannesburg Hospital Board

Since sprue was first described by VAN KETELAER in 1672 in the Dutch East Indies, and in 1766 by WILLIAM HILLARY in the Barbados, the aetiology of the disease has remained obscure

During the last war large numbers of troops were stationed in India and the Far East, and the incidence of sprue increased out of all proportion to the increased European population in these areas

The problem attained such proportions as to warrant the setting up of a Sprue Research Team by the India Command, and interest in the disease has been quickened by the published work of various members of this Research Team and also by the work of FRAZER (1946) on the physiology of fat absorption

Subsequent to the release of the prisoners of war in Japanese hands, the writer has had occasion to examine a considerable number of them both abroad and in the United Kingdom The striking fact was established that among the prisoners of war sprue did not occur

The object of this paper is to draw attention to this difference in the incidence of sprue among British troops in the field on the one hand and troops

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who were prisoners of war on the other and to discuss the aetiological factor or factors involved in the production of this difference.

MATERIAL

One hundred and twenty-seven released prisoners of war were examined and interrogated. These prisoners were all men who either were still suffering from the effects of their imprisonment or who were appearing before Medical Boards. They were all men who were or had been sick and therefore were not a representative cross section of all the prisoners. This, however does not invalidate the finding that not one of them had suffered from sprue. Brigadier J BENNETT the Consulting Physician to the Army who was himself a prisoner in Japanese hands, was approached, and he furnished a comprehensive report on the incidence of sprue among the prisoners under his medical supervision.

Other medical officers of various prisoner of war hospitals also stated their experiences concerning sprue among the prisoners. The Annual Medical Reports of the Prisoner of War Hospital Changi Camp (1942–45) were made available by the War Office Medical Directorate.

The figures regarding the incidence of sprue among British troops in the field were made available by the War Office Medical Directorate. Owing to the transference of administration in India, it was, unfortunately not possible to get figures in detail from the India Command but enough has been obtained to demonstrate the remarkable increase in the incidence of sprue among the troops in the Far East during the years 1942 to 1945

RESULTS OF INVESTIGATIONS

The striking rise in the incidence of sprue among our troops in the Far East during the last war is shown by the following figures, which give the number of men who were discharged from the army as being unfit for any type of further military service on account of sprue 1943, 6 1944 49 1945 196 1946, 73 1947 (January to August) 13

Out of 8,000 men invalided home from the Far East for medical as opposed to surgical reasons during 1944–1945 there were 1 000 cases of sprue. The number of men stationed in the Far East increased to almost double the initial number between 1942 and 1945 but this does not account for an increase in the discharge rate from the army due to sprue of from 6 to 196 over the same period. The conditions under which these men lived varied with their units and their tasks. Some were accommodated under reasonable conditions and ate an adequate and regular diet but in other cases the men were in huts or in the open during the humidity of the monsoon and were called upon to make great physical effort with rations often inadequate and irregular An example of the last type of case occurred among Chindits who were kept on a K diet for many weeks. The K diet is a compact emergency ration intended

to sustain the soldier for 2 days or possibly a week at the most, by which time it is hoped to supply him with fresh rations, and it is not intended to subsist men on this ration over long periods The Chindits lived on this ration without a change of diet, and during the first week they ate and enjoyed it After this, anorexia, diarrhoea, nausea and often vomiting after eating it developed This was followed by flatulent diarrhoea, and 3 weeks later sore tongues completed the picture of sprue 8 weeks after they had commenced, as healthy men, to exist on the " K " ration Eighty of these men subsequently reported sick as cases of malnutrition and, of these, 60 were suffering from sprue

In the case of the prisoners of war in Japanese hands, the story is entirely different They did not suffer from sprue One hundred and twenty-seven were examined personally and, although they had suffered from dysentery and from most of the known and some hitherto unknown deficiency syndromes, and many of them were still suffering from residua of these illnesses, not one had developed sprue in fact, only one of them had ever heard of the disease

BENNET (1947) describes diarrhoea among the prisoners in Singapore, a few cases of which developed a moderate anaemia of a normocytic and normochromic nature and a tendency to low levels of hydrochloric acid secretion, but states, " up to August, 1942, when I left Singapore, it could not be shown that these cases were in any way related to the sprue syndrome, and they were tentatively attributed to vitamin B_2 complex, possibly specifically one of nicotinic acid deficiency conditioned by the dysenteric state Cases of Wernicke's Encephalopathy had previously been common in the same group and had been controlled to some extent by the prophylactic administration of Marmite or yeast tablets of Japanese origin During the first 6 months of captivity in Singapore the diet consisted of unpolished rice 500 g , meat or fish 50 g , fresh vegetables 100 g , and its fat content was mainly represented by 5 g of cooking oil and an uncertain issue of 15 g of canned milk "

In Formosan camps the unpolished rice was augmented with 8 ounces of poor quality vegetable and the fat was represented by meagre quantities of vegetable oil, often averaging no more than 1 g per day per head Here, BENNET attributed the early cases of diarrhoea to mechanical irritation of the gut and hyperactivity of the gastro-colic reflex Although some cases developed sore tongue, only one case was diagnosed as sprue, and this diagnosis was supported mainly by the emaciation, which was out of all proportion to that seen in other cases with a similar history, and by the fact that the patient was refractory to dietetic treatment of the kind which was well tolerated and successfully employed in the other cases of diarrhoea

TAYLOR, CRUICKSHANK, MCFARLANE, HUSTON, GRAVES, HUNT and PHILLIPS (personal communications, 1947), all of whom were medical officers in prisoner of war camp hospitals, have stated in personal communications that their experiences were in agreement with those of BENNET and that sprue was practically unknown among the prisoners of war in the Far East

TAYLOR states that he remembers only one example of sprue among the prisoners in Changi camp, and this case was admitted to hospital with the disease before the commencement of captivity and remained in hospital until the prisoners were released. He concluded by stating "whether there were any more cases of sprue or not, it is certain that among the Singapore prisoners the disease was of the greatest rarity and I believe this also applies to the prisoners in Burma and Siam."

CRUICKSHANK, in 3½ years, saw two cases that could be clinically regarded as sprue, and both these were Dutchmen who had suffered from the disease before the war in Java. He states, "Watery diarrhoea was frequent in malnourished patients. The stools contained some pus cells no excess of fat microscopically and undigested food at times. At postmortem these patients were grossly wasted. The small intestine was diaphanously thin with marked congestion and atrophy of the valvulae conniventes some areas had a haemorrhagic appearance and in a small number of cases a plastic peritonitis associated with these areas. None of these, however could be regarded as sprue." The findings in the stool and the postmortem findings described by CRUICKSHANK certainly are not typical of sprue but appear to be due to changes of an irritative or inflammatory nature.

McFARLANE, who was in medical charge of prisoners in Thailand and at the Lahom Paton Allied Prisoner of War Hospital, where he had a dysentery wing of 1,200 beds, writes "Sprue was expected but never actually seen in Thailand and, as a differential diagnosis from amoebic dysentery it is scarcely worth mentioning" He states that all the cases presenting sprue-like symptoms were ultimately diagnosed pellagra.

The Prisoners of War Hospital in Changi Camp kept records which, considering the circumstances under which they were compiled, are remarkably complete. A study of the "Annual Medical Reports of the Prisoner of War Camp Changi, from 1942 until 1945 confirms that sprue was virtually not encountered under these conditions.

In the 1943–1944 Report, the following statement is made "Non-Specific Diarrhoea This was very common. In many cases it was probably due to vitamin B deficiency the bowel presenting an atrophic appearance though no cases of obvious sprue were encountered.

Thus, on the one hand, troops in India and Burma were developing sprue under the strain and hardships of war in very large numbers, whereas prisoners of war on the other were not affected by it at all, although their living conditions were as hard as, and in a great many cases worse than, those of the troops who were succumbing to the disease.

In one respect, however there was a great difference between the two groups and that was in the amount of fat and protein, especially animal fat and protein, in the daily diet. BENNETT (1946) tabulated the rations issued in No. 1 Prisoner of War Camp, Taiwan and the average nutritive value of these rations per head

daily From these tables it will be seen that in 1943 the mean meat content of the ration was 0 099 g daily, no fresh fish was issued, the daily amount of dried fish per head was 4 89 g, and only 0 69 g of oil was supplied The diet was made up with rice 410 g, soya beans 1 39 g, bean paste 4 169 g and bean sauce 1 5 g per man daily This was a daily average intake of only 5 29 g fat, 0 59 g animal protein and 42 99 g protein per man daily, the main bulk of the diet being 437 39 g carbohydrate In 1944 the situation improved considerably but the total fat intake only averaged 19 09 g daily, the total protein being 64 9 g, of which 3 1 g was animal protein, and the carbohydrate reaching an average of 639 39 g daily The protein and fat intake was therefore much less than that of Allied troops in the field, even when they were existing on emergency rations The "K" ration contains 90 g of protein, of which 70 g are animal protein, and 166 6 g of fat, of which 95 g are animal fat

Discussion

The present concept of sprue is that it is a conditioned deficiency of the vitamin B_2 complex, and LEISHMAN (1945) postulated that the condition necessary to precipitate this deficiency might be a change in the intestinal media upsetting the balance between biosynthesis and destruction of the B_2 complex in the intestine (BENESH, 1945)

KEELE (1946) found that glossitis and angular stomatitis may increase when the patient's diet contains adequate quantities of nicotinic acid and riboflavin and that these symptoms may remit without any additional intake of nicotinic acid or riboflavin He also found that acute sprue developed under jungle warfare conditions when the troops were subsisted on a well balanced diet

BLACK, BOUND and FOURMAN (1947) found that injections of nicotinic acid and riboflavin did not cause clinical improvement in sprue nor did they increase fat absorption

On the other side of the picture is the fact that prisoners of war, whose diet was deficient especially in animal fat and protein, did not develop sprue

MITCHELL and BLACK (1946) saw only one case out of 577 cases of malnutrition among released prisoners whom they examined

There is no evidence that protein metabolism is affected in the early stages of sprue—in fact, a positive nitrogen balance is the rule the oedema, which may be due to low plasma protein, is a late manifestation or occurs when the case is recovering under treatment It therefore seems that there are good grounds for regarding the fat intake as an important factor in the development of sprue and for postulating that, even though an individual be subjected to all the other conditions which favour the onset of sprue, he will not suffer from it unless he is ingesting a certain quantity of fat This hypothesis is supported by several well known features of the disease A very large proportion of early acute cases are successfully treated by dietary measures alone, and KEELE (1946) found that HAMILTON FAIRLEY's low fat, low carbohydrate and high

protein diet proved adequate in 67 per cent. of his cases without any liver other therapy Moreover native races do not suffer from sprue to the sa extent as Europeans, although cases have been described among them. Ke (personal communication, 1947) states that he has seen the condition in India particularly those who ate high fat diets, such as the Anglo-Indians, who eat diet which is practically European. This is in accordance with a perso impression gained in the West Indies from five cases of sprue seen in blacks British Guiana and two cases seen in Burmese in Rangoon. De Langen a Lichtenstein (1936) state that sprue affects the well to-do rather than t poorer classes, and it is well known that the fat intake depends largely on t financial condition of the individual. It may be argued that, as the prisone of war were living on a diet deficient in fat, it is hardly surprising that th did not manifest the signs of sprue. This argument would be valid if sever prisoners developed sprue when they returned to a normal fat intake after the release, thereby revealing that they were suffering from the disease but th it had been masked by a fat deficient diet, but this did not occur Many of the overate and developed transient diarrhoea, but sprue was an exceedingly ra finding among them.

It is possible that the ingestion of animal fats may be more favourab to the development of sprue than a diet in which vegetable oils form the bu of the fat content. Wintrobe (1942) noted that vegetable oil such as olive c may be well tolerated when fats of animal origin cause recrudescence of th diarrhoea and he quoted a seriously ill patient who was able to tolerate 1 ounc of olive oil daily Moreover Stannus (1947) suggested that the nature of th fatty acids in the diet might be a factor in determining the geographical di tribution of the disease. The symptoms of sprue fall into two distinct group The predeficiency symptoms begin insidiously as minor intestinal upsets abo which the patient is often very vague especially regarding the exact time of the onset, and later they merge into a fatty type of diarrhoea. If at this stage th patient avoids eating fat, the disease is arrested, but it appears probable th if he continues with a diet containing fat he will progress into the stage c vitamin deficiency and develop the typical cheilosis, sore tongue and anaemi and exhibit the typical picture of established sprue.

The breakdown products of the various fats and their ultimate fate in th body have not yet been elucidated, but Cooke, Frazer *et al.* (1948), investigatin the haematology of idiopathic steatorrhoea, postulate qualitative changes in fa absorption which may occur without any alteration in the total amount of fa absorbed. Such qualitative changes, which may represent a personal idiosyn crasy of the subject, are possibly of great importance in the aetiology of spru and may account for the conditioned deficiencies which are seen in the estab lished disease. The presence of undigested fat in the stool such as occurs i chronic pancreatitis lacteal obstruction and liver disease, is not accompanie

by symptoms of sprue and therefore the development of sprue would seem to depend on the presence of digested but unabsorbed fat in the intestine

The presence of these unabsorbed fatty acids in the intestine, especially if they display qualitative differences from the individual's normal metabolism, may be the factor which alters the intestinal media and conditions a B_2 complex deficiency as postulated by LEISHMAN (1945)

The conditions under which the prisoners of war lived, as opposed to those endured by the soldier in the field, constitute a mass experiment controlled by the regulations and rations of the Japanese and British armies respectively

The only difference that can be found in the conditions under which these two groups lived is one of diet, and particularly in the protein and fat intakes Further speculation as to the implications of the results of this experiment is not prudent, but it is intended to investigate further the influence of dietary fat on the vitamin utilization of the individual

SUMMARY

1 The enormous increase in the incidence of sprue among our troops in India and the Far East is contrasted with its virtual absence among prisoners of war in Japanese hands, who were living under identical or even greater hardship and were suffering from more dysentery and intestinal infections than the soldier in the field It is emphasized that these prisoners did not develop sprue when they were repatriated and ate a diet containing a normal fat content

2 The difference in the incidence of sprue between these two groups is attributed to the difference in dietary fat intake between the two groups it is suggested that qualitative changes in fat metabolism, which may be a personal idiosyncracy, may be important in determining the incidence of the disease

3 Sprue is described as comprising a pre-deficiency stage and a stage of conditioned deficiencies of the vitamin B complex It is concluded that intestinal infections are an important factor in precipitating the pre-deficiency stage but that the disease will not enter the deficiency stage if the diet contains no fat

REFERENCES

BENESCH, R (1945) *Lancet*, 1, 718

BENNET, J (1946) *Proc Nutrition Soc* 5 1 and 2 89

BLACK, D A K, BOUND, J P, & FOURMAN, L P R (1947) *Quart J Med*, 16, 99

CHANGI CAMP (1942–1945) *Medical Reports of P O W Hospital*, War Office, Medical Directorate

COOKE, W T, FRAZER, A C, PEENEY, A L P, SAMMONS, H G, & THOMPSON, M D (1948) *Quart J Med*, 65, 9

CRUICKSHANK, E (1947) Personal communication

DE LANGEN, C D, & LICHTENSTEIN, A (1936) *A Clinical Textbook of Tropical Medicine* 312 Batavia, Surabaya and Amsterdam G Kloff & Co

FRAZER, A C (1946) *Physiol Rev*, 26, 1

GRAVES, P R (1947) Personal communication

HILLARY, H. (1766). *Observations on the Changes of Air and Concomitant Epidemical Diseases in the Island of Barbadoes.* 2nd ed. London: L. Hawes, W. Clark and R. Collins.
HUNT, BRUCE. (1947). Personal communication.
HUTTON, J. (1947). Personal communication.
KEELE, K. D. (1946). *Brit. Med. J.* **2**, 3.
——— (1947). Personal communication.
——— & BOUND, J. P. (1946). *Brit. med. J.* **1**, 77.
LEVINSON, A. W. D. (1945). *Lancet*, **2**, 813.
MACFARLANE, L. (1947). Personal communication.
MITCHELL, J. B., & BLACK, J. A. (1946). *Lancet*, **2**, 855.
STANNUS, H. S. (1947). *Trans. R. Soc. trop. Med. Hyg.* **38**, 123.
TAYLOR, J. (1947). Personal communication.
WINTROBE, M. M. (1947). *Clinical Haematology.* 1st ed., page 300. London: Henry Kimpton.

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GAPS IN THE KNOWLEDGE OF YAWS

BY

C. J. HACKETT,

Director, Wellcome Museum of Medical Science, London

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The study of yaws appears to have suffered in several ways. It occurs in populations among whom full facilities for investigation are not often available. Its abundance and the ease of clinical diagnosis in endemic areas have called for mass treatment of large numbers of patients under conditions where the medical attendant can usually have little time for careful and prolonged observation. The lack of appreciation of its capacity for causing ill health and its economic significance probably accounts for the number of men who, having in the early part of their career investigated certain aspects of the disease, have soon abandoned the study of yaws for other fields.

The various aspects of yaws that still require investigation may be dealt with under a number of headings.

Aetiology.—A great advance in the cause of yaws was made when CASTELLANI, in 1905, described the *Treponema pertenue* in Ceylon. This followed the discovery by SCHAUDINN, in the same year, of the *Treponema pallidum* of syphilis. Their morphological similarity raised the problem of their identity which has only been made more difficult by the finding of an indistinguishable treponema in *bejel*, the non-venereal syphilis of bedouins (HUDSON, 1936 and 1937), and the discovery of *Treponema careteum* as the cause of pinta by SAENZ in 1938.

HUDSON (1946) is satisfied that there is only one treponema which, under varying epidemiological conditions, gives rise to different strains whose clinical manifestations may be those of syphilis, yaws, bejel or pinta. This view is not generally held by many who have studied yaws. STANNUS (1936) will not accept the arguments of BLACKLOCK (1933), who concludes that they are identical. These authors have depended upon epidemiological and clinical data. TURNER (1937) inoculated *T. pallidum* and *T. pertenue* intratesticularly into rabbits,

and concluded that the lesions produced were sufficiently constantly different over a long period to indicate that they are different organisms. TURNER *et al.* (1947), from a study of the immunity produced in rabbits after inoculations with *T pallidum*, *T pertenue* and *Treponema cuniculi*, concluded that the first two were more closely related to *T cuniculi* than they were to each other SCHÖBL and HASSELMANN (1932), after extensive inoculations with *T pallidum* and *T pertenue* into Philippine monkeys (*Cynomolgus philippinensis*), concluded that they were different The absence of syphilis in populations among whom the incidence of yaws is high has long been known and has been regarded as the immunization of adults resulting from childhood infection with yaws, but other factors are usually also present. These findings, however are not sufficient to give a decisive answer regarding the identity or difference of syphilis and yaws.

This surely merits a precise decision that can only be obtained by the study of the antigenic characters of the respective organisms by techniques in general use in bacteriology Before this will be possible it is necessary to obtain adequate suspensions of the treponemata. The use of the developing chick embryo as an experimental animal may provide this. There is need, also for a more suitable animal for experimental infections than those that have been used in the past.

Epidemiology.—It is generally acknowledged that yaws is acquired by an initial skin infection, usually in early life This could easily occur by direct contamination of skin abrasions by highly infective discharges from florid secondary lesions. Some primary lesions are doubtless due to this. Many of the few adult primary infections are in mothers nursing infected children the primary lesion is on the arm or breast, which are in obvious contact with infective lesions. The chances of infection in these cases must be enormous yet multiple primary lesions are almost unknown. The long time, 2 months, that *T pallidum* (HARRISON 1947) can survive, as tested by mobility out of the body providing they are kept moist, contrasts strongly with the findings of YASUYAMA (1928) that *T pertenue* survived, as tested by inoculation, outside the body at 28° C for only 30 minutes in saline and 2 hours in human serum. This calls for accurate knowledge which should be closely related to the high yaws incidence in wet countries and wet seasons, and the extent to which inanimate objects may be important in transmitting yaws.

KUMM and TURNER (1936) have called attention to the possible transmission of *T pertenue* by chloropid flies (*Hippelates pallipes*) in Jamaica. Why are so many primary yaws lesions on the lower part of the leg? This part in ill-clad peasant populations is subjected to considerable minor trauma. Do low flying insects play a part in this, or is infection carried on inanimate objects such as sticks, floors, etc R. D HARDING (personal communication) rightly calls ttention to the importance that climatic conditions inside huts at night may play in the transmission of yaws, especially in the wet season when folk

are forced under cover It is probable that congenital transmission does not occur but adequate observations have not been recorded (BAERMANN and SCHÜFFNER, 1912)

Geographical Distribution —This is fairly well known in a general manner, but differences of incidence in endemic areas have, as a rule, not been closely studied Such differences need correlation with climatic and geographical factors and the standard of life and hygiene of the populations concerned Is isolation the important factor in the maintenance of foci of yaws in India and elsewhere, or are other factors active ?

Pathology —It has been said that no autopsy has yet been carried out on a case of secondary yaws At least none has been recorded The distribution of the treponemata in the body, undoubtedly widespread, is quite unknown It is not known where the organisms remain during the quiescent periods between relapses

The changes present in the more frequent lesions have been studied (HALLENBERGER, 1916, FERRIS and TURNER, 1937, BOTREAU-ROUSSEL *et al*, 1937, MONTEL, 1944) There is need for detailed accounts of the pathological changes of all yaws lesions Those of palmar, plantar and bone lesions of both secondary and tertiary stages and tertiary skin lesions are particularly needed Pathological and clinical differentiation of secondary and tertiary yaws must proceed simultaneously

Clinical —Much is known of the clinical course of yaws (BOTREAU-ROUSSEL, 1938, MONTEL, 1944, HACKETT, 1946a), but continuous careful observation of the lesions of various stages is needed Although the secondary lesions of the skin should be well known, those of the tertiary stage are not so well defined The lesions of the palms and soles, both secondary and tertiary, are little known and are completely uncorrelated with the course of the disease This is obvious from the frequent use of such indefinite terminology as " inactive " and " non-infective " lesions No serious study has appeared since that of BAERMANN (1911) and, although this may serve as a basis, it leaves much to be desired The economic significance of these hand and foot lesions needs assessing

More information is needed on the character and evolution of the initial lesions Carefully conducted inoculations of human volunteers could add much to our knowledge of the incubation period and evolution of the early stages of yaws The study of yaws bone lesions has also been neglected (HACKETT, 1946b) Extensive clinical and radiographical studies made in Uganda in 1937–1940 have not yet been published

In many areas secondary lesions are most prevalent during the wet season (APTED *et al*, 1948) Consideration of the early age at which most yaws infections occur, and the rate of increase of the population and thus the number of susceptibles, shows that this seasonal incidence must be due largely to secondary relapses since there cannot be enough new infections to account for it (R D HARDING, personal communication) Careful clinical observation of yaws patients is often

impractical in the field because of the large amount of work to be done tropical practice. Under such conditions some types of lesions may be mi and thus thought not to occur in yaws. This is shown by the finding of bu mucous membrane lesions in 5 per cent. of secondary yaws cases in Uga (HACKETT 1946a). Some textbooks still quote their absence as one of points differentiating yaws from syphilis. The finding of these lesions attributable to adequate time making careful examination of the buccal ca possible. Frequent relapses during the first few years of the infection less frequently later are characteristic of secondary yaws. Is it a change the treponema itself or in the body of the host that initiates these relapses

There is need for thorough, at least 3-monthly surveys of random sample whole populations to find the incidence and seasonal variation of the various y lesions. However the opportunity for these has probably already gone a surveys could not, on humanitarian grounds alone, be carried out with chemotherapy which would change the picture. The manifestations of ya in one country need comparison with those in other countries, but the l of careful studies and descriptions prevents this. In this respect the effect malnutrition and other infections upon the course of yaws should be studied.

A careful search is needed in yaws areas, where syphilis does not occ for late heart and central nervous system lesions.

Diagnosis.—In yaws endemic areas, clinical diagnosis is not usually diffic especially if syphilis is absent. So wide is the range of yaws lesions that th are probably none that might not be seen in syphilis. However no satisfacto record have yet appeared of epiphyseal osteochondritis in infants, congeni infection, visceral gummata and heart, nervous system or eye lesions in ya but the present knowledge of yaws is limited to skin and bone lesions.

The finding of treponemata and of positive non-specific serological re tions are of no value in differentiation from syphilis. Non-specific serologi reactions may be valueless in assessing the yaws origin of any lesion since in highly endemic yaws population the sera from over 80 per cent. (HACKETT 1947) of apparently healthy subjects may give positive Kahn reactions. T incidence of positive reactions in non-specific serological tests in the absen of treponemal infection in tropical populations needs investigation. Althou full knowledge of yaws will allow a fairly accurate assessment of the treponem or even yaws, origin of a lesion, the adequate differentiation between yaws an syphilitic lesions awaits the discovery of satisfactory specific serological tests.

Treatment.—The efficiency of chemotherapy in syphilis is generally assesse by prolonged serological observation. Similar studies in yaws are chief restricted to secondary lesions and limited to observation periods of at mo 2 years. Results of therapy based on clinical observation alone are of som value in assessing the preventive effect but must be continued over many year Serological observation would reduce this period. Important work has bee reported by the Jamaican Yaws Commission (1936) and ARTED *et al.* (1945)

as regards arsenical and bismuth preparations, and DWINELLE *et al* (1947) report an excellent study of the efficacy of penicillin It is essential that the composition of preparations and the metallic content of bismuth salts used should be included in all reports of treatment so that accurate comparison will be possible

Further study is required to establish the minimum amount of a drug or combination of drugs (*a*) to reduce the relapse of infectious lesions to a level low enough for ordinary dispensary treatment to keep the disease under control, and (*b*) to reverse positive serological reactions In some areas the dose required to produce clinical cure is very close to that required to produce serological reversal (VAN NITSEN, 1944) One or two doses of an arsenical preparation have been observed to produce serological reversal in some cases of secondary yaws

In assessing the results of treatment the stage of the disease, *e g*, primary lesions still present, first eruption of secondary lesions with or without primary lesions, relapses and duration of infection, tertiary lesions, and the season of the year in which the clinical observations were made, must be taken into account.

Prognosis—The menace of yaws as a cause of suffering is stressed by VAN NITSEN (1944) but is not generally realized Advanced gangosa and advanced extensive tertiary bone and skin lesions with contractures about joints and muscular wasting, present saddening pictures The economic disability arising from palm and sole lesions in young adults is generally under-estimated

Will a community among whom yaws is prevalent and syphilis is rare suffer severely from the latter if the former is eradicated? VAN NITSEN (1944), from observations in the Belgian Congo, says that no high incidence of syphilis occurred when yaws was greatly reduced It is important that this question be truly answered Careful observation is needed since other changes also take place in a community while yaws is being eradicated

Prevention—At present mass treatment is the immediate preventive measure in practice (HARDING, 1949) In many areas where anti-yaws treatment is readily available and free, a very remarkable reduction in yaws has been observed in a few years The prevention of the transmission of yaws may prove to be so straight-forward that simple and inexpensive hygienic measures may well be very effective The implementation of these measures will consist almost entirely in the education of the population at risk This must come in time, but the necessary steps to hasten its achievement call for urgent planning and action

SUMMARY

A complete description of yaws has yet to be written. Many gaps in the knowledge of yaws exist To fill them requires work by bacteriologists, epidemiologists, pathologists, clinicians and hygienists Some of this work

must be carried out in laboratories but much of it must be done in the field. It is hoped that the indication of what knowledge is still needed may stimulate those with the necessary opportunities to plan and undertake the studies required. Probably tens of millions of tropical people are infected with yaws thus its investigation is of more than academic interest.

REFERENCES

AFRID, J HARDING, R. D & GORDON M. (1948). *Trans. R. Soc. trop. Med Hyg* 42, 55.

BAERMANN, G (1911). *Arch Schiff -u Tropenhyg* 15 Beiheft 6 334

——— & SCHÜFFNER W (1912). *Ibid* 16 Beiheft 4 48.

BLACKLOCK, D B (1933). *Trop Dis Bull.*, 30 739

BOTREAU ROUSSEL, J M. (1935) *Clinique chirurgicale des pays chauds* 819 Paris Masson et Cie

——— FARGES & GAUTHIER-VILLARS (1937). *Ann. Anat path. méd.-chir* 14 711

DWINDELLE, J H. SHELDON A. J REIN C R., & STERNBERG, T H. (1947). *Amer J trop Med.*, 27 633

FERRIS, H W & TURNER, T B (1937) *Arch Path.*, 24 703

HACKETT C. J (1946a). *Trans R. Soc trop Med. Hyg* 40 206

——— (1946b). *Trop Dis Bull.* 43 1091

——— (1947). *Brit med. J* 1 88

HALLENBERGER (1916). *Arch. Schiffs-u Tropenhyg* 20 Beiheft 3 155.

HARDING, R. D (1949). *Trans. R. Soc trop Med. Hyg* 42 347

HARRISON L. W (1947). *Lancet* 2 984

HUDSON E. H. (1936). *New Engl J Med* 215 392.

——— (1937). *Trans. R. Soc trop Med. Hyg* 31 9

——— (1946). *J trop Amer Med* 26 153

JAMAICA YAWS COMMISSION (1936) *Annual Report* Kingston Government Printing Office

KUMM, H. W & TURNER, T B (1936). *Amer J trop Med.*, 16 245

MONTEL, R (1944) *Bull Soc Path exot* 37 137

SCHÖBL, O & HASSELMANN C. M (1932). Beiheft 2, *Arch. Schiff -u. Tropenhyg* 36

STANNUS, H S (1936) *Yaws-syphilis Empire Social Hygiene Year Book* 577 London George Allen & Unwin, Ltd.

TURNER, T B (1937) *Amer J Hyg* 25 477

——— McLEOD C & UPDYKE, E L. (1947) *Amer J Hyg* 46 287

VAN NITSEN R. (1944) *Le Pian*, Memoire publie par l'Institut Royal Colonial Belge (Section des Sciences naturelles et medicale) Tome XIII

YASUYAMA, K. (1928) *Philipp J Scienc* 35 333

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OBSERVATIONS ON HAEMOGLOBIN VALUES IN AFRICAN CHILDREN*

BY

E A BEET,

Medical Officer, Colonial Medical Service, Northern Rhodesia

Most haematological research in Africa on the native population has been carried out by workers in laboratories and hospitals upon individuals who have been unwell and who, therefore, are not representative of the African in normal health Such work is of fundamental importance in determining the types of anaemia present in any group but, as BURKE-GAFFNEY (1948) points out, pathological research must remain without an adequate foundation until acceptable physiological standards are arrived at, and " haematological and other normals in Africans are of prime importance " The lack of figures for haematological normals in Africans is to be deprecated and is shown by the table given by KENNEDY (1939), in which are tabulated haemoglobin standards determined by various authorities In this table are quoted 18 authors working in eight countries of North and South America, Europe, Australia and Asia, but not one example is given from the Continent of Africa

The inevitable result of this absence of basic data is that workers in Africa have had to compare their results with standards obtained elsewhere, in the case of the British Colonies of East and West Africa from England To assume that English standards are applicable to the African in his natural habitat is most unwise, and any conclusions drawn from such an assumption may be quite erroneous

The object of this paper is to record the results of haemoglobin determinations performed on 630 rural African school children of Northern Rhodesia who were examined at their schools in the native reserves This work was part of a health survey carried out by the writer on Lala school children in 1947–48

The Lala are a Bantu tribe who live in the Serenje and Mkushi Districts of the Central Province of Northern Rhodesia, the area so far visited covers 9,000 square miles, the average density of the population must be well under seven persons per square mile Sixteen schools have been visited which are scattered throughout the area, seven of these are accessible by road, three are within 15 miles of a motor road, and the remaining six have to be visited on foot with native carriers , the most isolated school was on the Luangwa River, about 80 miles from the nearest road All the schools in the district have not

* I am indebted to the Director of Medical Services, Northern Rhodesia, for permission to publish this paper

malnutrition can cause anaemia is without question, that malaria can also produce anaemia is an established fact that schistosomiasis and hookworm infestation can produce anaemia is generally accepted but there appears to be little in the way of definite proof of this

All the children in this survey have had blood smears examined for malaria parasites, and their stools and urines for helminths. The specimens from each child were examined on one occasion only Thick blood smears were stained

TABLE II.—HAEMOGLOBIN LEVELS AND HEIGHT GROUPS.

Hb in grammes per 100 c.c.	Heights in inches.						
	45 and below	—51	—54	—57	—60	—63	63
	Approximate age in years.						
	6 and below	—9½	—11	—13	Over 13		
8 00— 8	1	3	3	0	0	0	0
— 9·43	[illegible]		0	1	1	0	0
—10 15	1	[illegible]	7	7	4	1	[illegible]
—10·87	6	[illegible]	[illegible]	[illegible]	3	2	0
—11 85	3	17	21	[illegible]	18	4	1
—12 [illegible]3	3	14	[illegible]	3[illegible]	27	7	2
—1[illegible] 03	7	16	[illegible]	[illegible]	32	[illegible]	3
—13 63		[illegible]	18	[illegible]	1	18	[illegible]
—14 33	1	3	10	12	16	[illegible]	12
—18 03	[illegible]	1	3	[illegible]	7	2	2
—15 78	0	1	[illegible]	1	[illegible]	3	2
—16 43			[illegible]	[illegible]		[illegible]	1
	58	[illegible]	143	174	1	[illegible]	[illegible]
Hb means grammes per 100	11 6	11 7	12	12 3	1 3	13	13 7

χ^2 = 163 783 and therefore p is less than 0 01

N B —One case has been excluded owing to faulty length record, making total of 620.

with Field's double stain the urines were centrifuged with hand centrifuge and the stools were suspended in a saturated solution of magnesium sulphate and the supernatant fluid was searched for ova.

The stools of 193 children were also examined for *Schistosoma mansoni* ova using a suspension of faeces in normal saline. This parasite was found on seven occasions but, as the number is so small, mansoni infections have not been included in the table given below Urinary schistosomiasis, due to *S haematobium* is invariably meant when reference is made to schistosomiasis in this paper

Hookworm Infection

Table III gives the figures for those children proved to harbour hookworms (almost certainly *Necator americanus*), and those whose stools were negative

TABLE III —HAEMOGLOBIN VALUES FOR HOOKWORM INFECTED AND NON-INFECTED CHILDREN

	Number	Per cent with hookworm	Hb mean in gm per 100 c.c.	Range in gm per 100 c c
With hookworm	299	47	12 3	8 4 to 15 4
Without	331		12 3	8 4 to 16 1

It is tempting to assume that the identical means for the haemoglobin values for these two groups show that hookworm infection is not a cause of anaemia in these children, thus confirming the work of DICK and MCCARTHY (1946), who showed that there was no difference in the red cell counts and haemoglobin levels in 64 askari with hookworm and 15 whose stools were negative The stools from each child were examined once only, unquestionably if it had been possible to examine the children on more than one occasion many more infected children would have been found Even with one examination only, the hookworm incidence is 47 per cent , with so high an incidence, many subjects must have been included in the non-infected group who would have been proved to be positive on a further examination If hookworm infection does cause an anaemia, the inclusion of infected cases in the non-infected group would inevitably lower the mean haemoglobin level of the latter This may have happened in this series of cases where the true hookworm incidence is probably in the region of 75 per cent

Commenting on DICK and MCCARTHY'S paper, WRIGHT (1946) advises caution in accepting the idea that poor diets are the main cause of an apparent hookworm anaemia, and that hookworm infestation itself is not a cause of low haemoglobin levels

As far as this present survey is concerned it is wisest to adopt the attitude that, as there is a very high hookworm infection rate, and also a low haemoglobin level in these children, hookworm infestation may well be in part the cause of the prevalent anaemia Unfortunately, hookworm infection is prevalent throughout the district, and it has not been possible to compare a hookworm-free area with one having a high infection rate

Urinary Schistosomiasis

Table IV compares children with *S haematobium* ova in their urines, and those whose urines were normal

There is a significant difference in the mean values for infected and non-infected children as the difference is nearly eight times the standard error of the difference between the means This shows that children with urinary schistosomiasis have definitely lower haemoglobin levels than non-infected children

TABLE IV.—HAEMOGLOBIN LEVELS OF BILHARZIA INFECTED AND NON-INFECTED CHILDREN.

Haemoglobin grammes per 100 c.c.	Children with schistosomiasis.	Children without schistosomiasis.	Total.
8·03—8·75	6	3	9
—9·43	0	2	2
—10·15	11	13	[illegible]
—10·85	16	15	[illegible]
—11·55	44	62	106
—12·25	38	91	[illegible]
—12·95	27	107	134
—13·65	19	82	101
—14·35	7	57	64
—15·05		21	[illegible]
—15·75	0	6	6
—16·45	0	1	1
	170	460	630
Haemoglobin mean gm. per 100 c.c.	11·4	12·3	12·3

Difference between means = 0·9 gm.
Standard error of the difference between means = 0·117 gm.
Percentage with schistosomiasis = 27

It has already been demonstrated in Table II that the younger the children the lower are the haemoglobin levels if the average age of the children with schistosomiasis is lower than that of the uninfected children this would account for the low haemoglobin levels in the former group In Table V are compared the heights of uninfected and infected children.

TABLE V.—SCHISTOSOMIASIS AND HEIGHTS.

	Number	Mean height inches.	Range in inches.
With schistosomiasis	166*	54·96	Under 45 to over 63
Without	460	53·44	45 63

Reference footnote to Table II.

From Table V it is apparent that the mean height of the children with schistosomiasis is less than that for uninfected children the difference however is too small to account for the low haemoglobin mean of the children with schistosomiasis It can therefore be concluded that the low haemoglobin level of the infected children is probably due to the infection as, apart from this, they are comparable with the uninfected children

Schistosomiasis is not evenly distributed through the district surveyed consideration of this point gives another method of investigating the relationship

between schistosomiasis and anaemia in these children The Lala inhabit two distinct types of country, the majority live in the region of the Zambesi-Congo watershed at an altitude of 4,000 to 5,000 feet, the rest of the people live at a much lower altitude, of about 1,500 to 2,000 feet, along the Lukusasi River, which receives the streams and rivers running down the Zambesi side of the watershed The schistosomiasis incidence of the north-eastern part of the highlands area is very low (5 per cent), that of the south-western part of the highlands area and all the low-lying valley area is high (41 and 49 per cent respectively) The data for these areas is given in Table VI

TABLE VI —DISTRIBUTION OF SCHISTOSOMIASIS

	N E highlands	S W highlands	Valley
Total children	277	160	193
Children with schistosomiasis	13	78	79
Percentage with schistosomiasis	5	49	41
Mean Hb in gm per 100 c c	12 9	12 1	11 5
Standard deviation in gm	1 2	0 9	1 2
Range in grammes	8 4 to 16 1	9 8 to 14 7	8 4 to 14 0

The hookworm and malaria incidences are similar for all three areas The valley children live under very different physical conditions compared to the highlands children, their relatively low haemoglobin level is in part due to this, however, it is considered that the high schistosomiasis rate is also a factor in causing their anaemia The interesting point is that the mean haemoglobin level of the children from the S W portion of the highlands is low compared to the mean of those living in the N E part, the higher upper point in the haemoglobin range of the latter group is also to be noted Apart from the high incidence of schistosomiasis in the children from the south-west, the conditions under which these children live are very similar in all respects

It would thus appear that urinary schistosomiasis is a definite factor in the causation of anaemia in Lala school children

Malaria

Table VII compares the haemoglobin means of children with and without malaria parasites in thick blood smears The children have been divided into two groups, those who were examined during the rainy season (November to April), when the transmission of malaria is at its highest, and those who were seen in the dry season (May to October), when the transmission of malaria is at its lowest In the first group are children from the N E highlands area —this accounts for the high haemoglobin means here, and in the second group are children from the rest of the highlands and the valley area

TABLE VII.—HAEMOGLOBIN LEVELS AND MALARIA.

		Total.	Percentage with malaria.	Hb mean gm. per 100 c.c.	Range gm. per 100 c.c.
Group I Nov. to April	Children with malaria	137	49	12·0	8·4 to 15·4
	without	140	—	13·1	8·1 to 16·1
Group II May to Oct.	Children with malaria	177	50	11·7	8·4 to 14·7
	without	176		11·9	8·4 to 14·0

The difference in the means in each of the two groups is not of any statistical significance.

As in the case of hookworm infection the high incidence of malaria probably means that many cases have been classified as negative which would have been found to be positive on subsequent examinations. The argument and conclusions made in the sub-section on hookworm infection apply to this sub-section.

As only thick smears were examined, it has not been possible to give accurate figures for the species of plasmodia found in this survey. *Plasmodium falciparum* is the commonest parasite but *P. vivax* seems to occur more frequently than is usual in East Africa, where vivax infections are only infrequently seen, (BROCK, 1945). *P. malariae* was seen on a few occasions, but *P. ovale* was never diagnosed.

Multiple Infections

In Table VIII comparison has been made between children with urinary schistosomiasis only and those with schistosomiasis malaria and hookworm infection.

TABLE VIII.—HAEMOGLOBIN LEVELS AND MULTIPLE PARASITIC INFECTION.

	Number	Hb mean in gm. per 100 c.c.	Range in gm. per 100 c.c.
Children with schistosomiasis only	49	11·6	8·4 to 14·0
Children with schistosomiasis, malaria and hookworm	4	11·7	8·4 to 14·0

It is interesting to note that children with schistosomiasis are not more anaemic when they are burdened with an added hookworm and malarial infection.

Enlarged Spleen.

Table IX shows how children with a palpable spleen compare with children without a palpable spleen.

TABLE IX —HAEMOGLOBIN LEVELS AND PALPABLE SPLEEN

	Number	Percentage with palpable spleen	Hb mean in gm per 100 c c	Range in gm per 100 c c
With palpable spleen	224	36	11 8	8 4 to 14 7
Spleen not palpable	406		12 5	8 4 to 16 1

Difference between means = 0 7
Standard error of difference between means = 0 11

The lower mean haemoglobin value of the children with a palpable spleen is statistically significant as the difference between the means is over six times the standard error of the difference

As in the case of schistosomiasis, this low value for children with a palpable spleen may be due to a higher spleen rate for children in the younger age groups That this is the case is shown in Table X, where the children are divided into height groups The mean height of the children with a palpable spleen is 3 inches less than that for the children in whom no spleen was palpable This difference is sufficient to account for the lower haemoglobin mean of the former group

TABLE X.—PALPABLE SPLEEN AND HEIGHT GROUPS

Height, inches	Approximate age in years	Number with palpable spleen	Number without palpable spleen	Total	Spleen rate as percentage
Below 48	Below 6	14	14	28	50
—51	— 9½	36	32	68	53
—54	—11	60	85	145	42
—57	—13	59	115	174	34
—60	13+	39	89	128	30
—63		13	43	56	23
Over 63		3	27	30	10
		224	405	629*	
Mean height		53 3 inches	56 3 inches		

* Reference footnote to Table II

As the age increases the spleen rate decreases This fact may account for the low haemoglobin means for the lower age groups as demonstrated in Table II

Summary

(1) The mean haemoglobin value for 630 Lala school children of Northern Rhodesia was found to be 12·3 ± 0·05 grammes per 100 c.c. blood. The means for the sexes were similar.

(2) The mean haemoglobin value progressively increases from the youngest to the eldest age group.

(3) The mean haemoglobin value for children with hookworm infection was 12·3 grammes per 100 c.c.; the mean for children with negative stools was also 12·3 grammes per 100 c.c.

(4) The mean haemoglobin value for children with urinary schistosomiasis was 11·6 grammes. The mean for children with normal urines was 12·5 grammes per 100 c.c.

(5) The mean haemoglobin value for children with malaria examined during the rains was 12·9 grammes; for children with negative blood smears the mean was 13·1 grammes per 100 c.c. In a group of children examined in the dry weather the means were 11·7 and 11·9 grammes per 100 c.c. respectively.

(6) The mean haemoglobin value of children with a palpable spleen was 11·8 grammes per 100 c.c. and for children without a palpable spleen was 12·5 grammes per 100 c.c.

(7) The mean haemoglobin values for children with and without sicklaemia were 12·5 and 12·3 grammes per 100 c.c. respectively.

(8) The effect on the Lala diet on haemoglobin levels is briefly mentioned.

REFERENCES

BAKER-JONES, E. (1942). *Trans. R. Soc. trop. Med. Hyg.* **36**, 99.

BETT, E. A. (1946). *E. Afr. med. J.* **23**, 75.

——— (1947). *Ibid.* **24**, 12.

BROCK, J. F. (1945). *E. Afr. med. J.* **22**, 334.

BURKE-GAFFNEY, H. J. O'D. (1948). *Trop. dis. Bull.* **45**, 640.

DICK, G. W. A. & MCCARTHY, D. D. (1946). *E. Afr. med. J.* **23**, 19.

GELFAND, M. (1944). *The Sick African.* 1st Ed., 209. Cape Town: Stewart Printing Co.

HILL, A. BRADFORD. (1945). *Principles of Medical Statistics.* 4th Ed. London: Lancet, Ltd.

KENNEDY, W. P. (1939). *Trans. R. Soc. trop. Med. Hyg.* **32**, 511.

MANSON-BAHR, P. H. (1935). *Manson's Tropical Diseases.* 10th Ed., 63. London: Cassell & Co.

PRESTON, B. (1948). Personal communication.

SHELDON, W. (1943). *Diseases of Infancy and Childhood.* 4th Ed., 6. London: Churchill.

STEPHEN, J. D. (1948). *Trans. R. Soc. trop. Med. Hyg.* **41**, 829.

WRIGHT, F. J. (1946). *E. Afr. med. J.* **23**, 124.

WHITBY, L. E. H. & BRITTON, C. J. C. (1947). *Disorders of the Blood.* 5th Ed., 48. London: Churchill Ltd.

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INSULIN-HYPERSENSITIVITY IN PELLAGRINS AND ITS SIGNIFICANCE

BY

FR MAINZER

From the Anglo-Swiss Hospital, Alexandria, Egypt

Some years ago (MAINZER, 1939) we showed that in pellagrins, after a small dose of insulin, an important decrease of the blood-sugar occurs, often followed by severe manifestations of hypoglycaemia The subsequent rise of the glycaemia, as observed in normal beings, may be insufficient or not take place at all In a second paper (MAINZER and KRAUSE, 1940) we reported that the pellagrous insulin-hypersensitivity remains unchanged in spite of improvement or clinical cure of the disease

Tentatively, we attributed the disturbed regulation of the blood-sugar to cortico-adrenal damage A number of clinical and experimental findings seemed to support this interpretation

(*a*) Pathologists have repeatedly noted severe adrenal damage in pellagrins (ASCHOFF, 1933, FROBOESE and THOMA, 1933, FROBOESE, 1934), in some instances reporting on extensive observations (HERZENBERG, 1935)

(*b*) In patients with adrenal insufficiency (Addison's disease) or with anterior pituitary insufficiency (Simmond's disease), similar disturbances of carbohydrate metabolism are present, *e g*, occasionally a decrease of the fasting blood-sugar, regularly hypersensitivity to insulin (MARANON, 1925, UMBER, 1926, LUCKE, 1933, FRASER *et al*, 1941) Moreover, some clinicians have emphasized the striking clinical analogies between pellagra and adrenal insufficiency (FINOTTI and TEDESCHI, 1902, THANNHAUSER, 1933a, 1933b), which, as we know from personal experience, may render the differential diagnosis very difficult

(*c*) Experiments on pigeons and rats carried out in the pioneer period of vitamin research have shown the regular occurrence of gross anatomical and histological damage of the adrenals in thiamin deficiency and lack of the heat-stable fraction of vitamin B, not yet differentiated at this time (FUNK, 1919, McCARRISON, 1921, VERZAR and V BEZNAK, 1923, FINDLAY, 1928)

(*d*) Biochemical findings on the metabolism of fat absorption by the intestinal mucosa were interpreted by the investigators themselves with reference to the disturbances of absorption in adrenal insufficiency and pellagra (VERZAR, 1935, VERZAR and McDOUGALL, 1936) The authors found an impairment of phosphorylation processes—an important step in intestinal absorption and in intermediary carbohydrate metabolism—in adrenal insufficiency as well as in "B_2 vitamin" deficiency It is true, the interpretation of these experiments has since become controversial, because others (MARAZZI and GAUNT, 1939, FERREBEE, 1940) found not only cortical hormone, but also ingestion of sodium salts effective in restoring the intestinal absorption in adrenalectomized animals

On the other hand, some new research work has strengthened the experimental basis of our earlier conception

Since 1939, several investigators have reported an increase in weight of the adrenals, and necrosis and haemorrhage of the cortex as a regular occurrence in pantothenic acid deficiency (DAFT and SEBRELL, 1939, MILLS *et al*, 1940, MORGAN and SIMMS, 1940a

Experiment		Number of individuals	Number of experiments	Blood-sugar: Initial level, mg. %: Mean	Standard deviation	Maximum	Minimum	Minimal level, mg. %: Mean	Standard deviation	Maximum	Minimum	Decrease, mg. %: Mean	Standard deviation	Maximum	Minimum	Surface-area, mm.: Mean	Standard deviation	Maximum	Minimum
Normal humans	A	10	10	94	±8	100	84	72	±11	88	61	23	±9	38	9	325	±100	476	141
Pellagrins	B	—	99	83	8	112	58	48	±14	82	11	35	±13	74	14	721	±270	1 583	284
	C	33	—	88	6	103	6	45	±12	70	14	40	±11	69	14	50	±44	1 229	114

TABLE II

INSULIN BLOOD-SUGAR CURVES IN PELLAGRINS AND NORMAL HUMANS: DISTRIBUTION OF THE MINIMAL LEVELS AND LEVELS AFTER 5 HOURS.

Blood-sugar mg.	99–90	89–80	79–70	69–60	59–50	49–40	39–30	29–20	19–10	Interrupted for hypoglycaemia	Diabetics.	Total.
A Minimal levels, mg. (a) *Normal humans* number	0	4	1	4	1							
(b) *Pellagrins* number	0	0	7	13	15	[illegible]	[illegible]	10		(23)*†	(3)	99
Per cent		0	7	13	18	[illegible]	24	10	2	—	—	100
B Levels after 5 hours, mg. (a) *Normal humans* number	4		0									
(b) *Pellagrins* number	6	15	7	9	7	5	2			(23)*	(3)*	71
Per cent		1	20	12	10	7	3			—	—	100

* Omitted from calculations. † Tabulated as minimal levels.

In Tables I, II and III the results in pellagrins and normal humans are summarized Tables II and III are illustrated by the Figs 2, 3 and 4

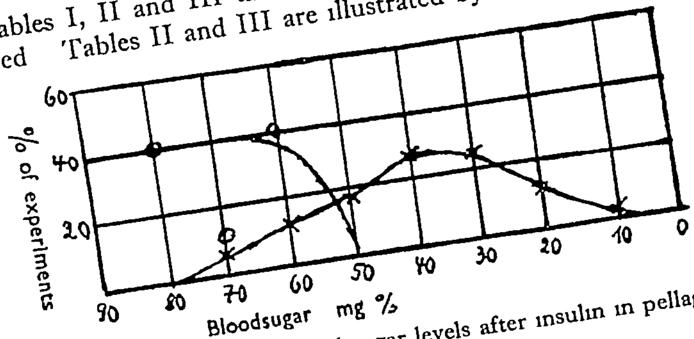

FIG 2—Distribution of minimal blood-sugar levels after insulin in pellagrins and normal humans

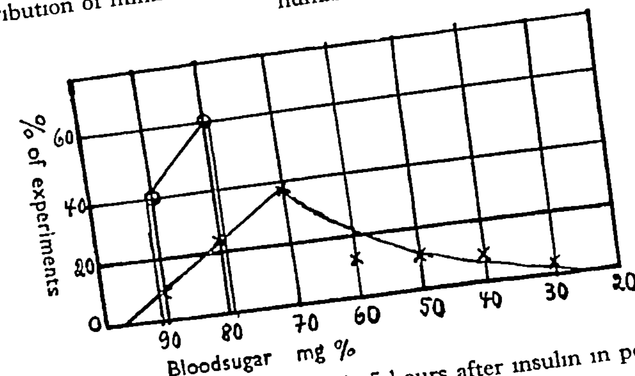

FIG 3—Distribution of blood-sugar levels 5 hours after insulin in pellagrins and normal beings

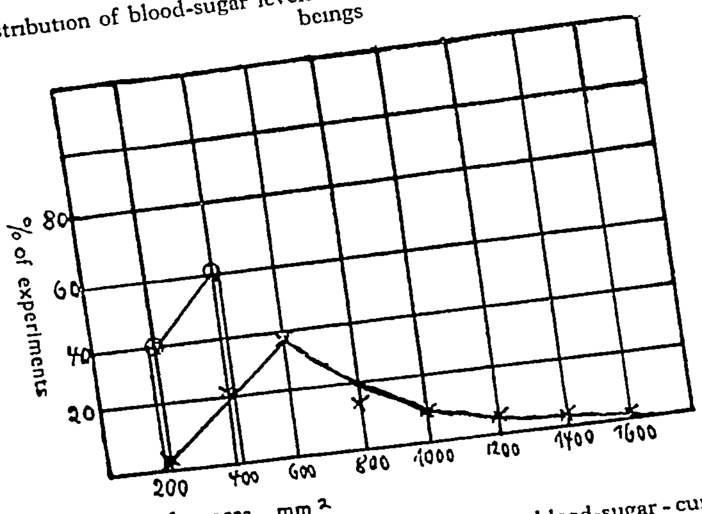

FIG 4—Distribution of the surface-areas of the insulin-blood-sugar-curves in pellagrins and normal beings

TABLE III.

INSULIN BLOOD-SUGAR CURVES IN PELLAGRINS AND NORMAL HUMANS. DISTRIBUTION OF THE FIGURES FOR THE SURFACE-AREA.

Mm²	101–300	301–500	501–700	701–900	901–1100	1101–1300	1301–1500	1501–1700	Diabetics.	Others	Total.
(a) *Normal humans*											
Number	4	6	0								
(β) *Pellagrins*											
Number	2	20	24	13	14	8	3	1	(3)*	(3)*	89
Per cent.	2	21	27	14	15	7	3	1	—	—	100

* Omitted from calculations.

The results in healthy people suggest to define the normal range of the insulin effect (under the conditions of the present experiments) at a minimal blood-sugar level of 60 mg. per cent. The mean blood-sugar level at the height of the insulin effect is as low as 45 mg. per cent. The strongest insulin action was noted at 11 mg. per cent. in a pellagrin, which attained in three tests blood sugar levels below 20 mg. per cent.

The fasting blood-sugar level of pellagrins was mostly near the lower limit of the normal range, sometimes even below it (in one case, 56 mg. per cent.). The mean value (85 mg. per cent.) was distinctly lower than in healthy people (94 mg. per cent.). Because of the low initial level, the decrease in pellagrins (mean value 39 mg. per cent.) differs from the result in normal humans (mean value 23 mg. per cent.) to a lesser extent than would be expected from the figures of the minimal levels.

For the total surface-area obtained by graphic integration of the curves, however, the difference of the figures for pellagrins and for normal humans is greater than for the respective minimal blood sugar levels. In pellagrins the mean insulin effect as represented by the blood sugar decrease (39 mg. per cent.) amounts to roughly 170 per cent. of the mean decrease in normal beings (23 mg. per cent.), but, if calculated as surface area (721 mm.²), it amounts to not less than 222 per cent. of the mean normal figure (325 mm.²). This difference emphasizes the fact that the insulin hypersensitivity is even more manifest by the absence or insufficiency of the compensatory blood-sugar adjustment than by the excessive scale of its primary decrease.

As evidenced by Figs. 1 to 3, the areas of pellagrins and normal humans are overlapping to a large extent only for the minimal blood-sugar levels (Fig. 2); in fact, 36 per cent. of their total number in pellagrins are within the limits of the normal range; the respective figures are 29 per cent. for the levels after 5 hours and 23 per cent. for the surface-areas.

With a very few exceptions, normal insulin-sensitivity in pellagrins, where present, could be reproduced quantitatively in repeated experiments. Insulin-hypersensitivity, even of an extreme degree, once present in a patient, could likewise be reproduced. Table IV summarizes the figures obtained in such a case (J B)

TABLE IV

CONSTANCY OF INSULIN-HYPERSENSITIVITY IN A PELLAGRIN

Date	Minimal blood-sugar level mg %
25 1 38	46
15 2 38	44
5 3 38	42
14.2 40	43

In one pellagrin (S B), during a first attack the insulin-sensitivity was within normal range (three experiments), later on, during two relapses, a constant hypersensitivity was noted (seven experiments), which had developed within 3 months

There was no correlation between insulin-hypersensitivity and the presence or intensity of other pellagra manifestations. Correlation seemed to exist, however, with the duration of the illness. In fact, in two cases of acute pellagra with a short previous history—the only two cases of this kind observed—the insulin-sensitivity was within the normal range. In all pellagrins the insulin-hypersensitivity—where present in the beginning (13 patients)—remained unchanged in spite of clinical improvement or cure

Observation and treatment lasted generally from 4 to 10 weeks and, therefore, it remains open to question if normal sensitivity could have been restored by a more prolonged intensive nutritional treatment

As shown by FLETCHER and CAMPBELL (1932) at the beginning of the insulin era, two phases of the hypoglycaemic reaction can be discerned, the first phase is characterized by manifestations of autonomic origin: acceleration of the pulse rate, sensation of hunger, profuse sweating, muscular twitching and vasomotoric disturbances. These phenomena are followed if the hypoglycaemia persists or progresses, by central nervous symptoms of a more serious character: paralysis of the external ocular muscles, excitation, disorientation, delirium and finally coma

During the experiments on normal humans, hypoglycaemic symptoms other than pulse acceleration, were not observed. In pellagrins, phenomena o[f] autonomic origin were noted in 13 instances without subsequent central nervou[s] disturbances. The number is probably too low, since not all the protoco[ls] are explicit about this point

... four experiments in pellagrins, nearly a quarter of the to

number had to be interrupted prematurely by glucose ingestion, intravenous or oral, because of severe cerebral manifestations. It should be stressed that in these cases hypoglycaemic phenomena of autonomic origin did rarely precede the cerebral signs or were very slight.

Table V gives a survey of the experiments interrupted for hypoglycaemic manifestations.

TABLE V

EXPERIMENTS INTERRUPTED BECAUSE OF HYPOGLYCAEMIC MANIFESTATIONS.

Interrupted (minimal blood-sugar level within 120 minutes.	Interrupted (minimal blood-sugar level within 150 minutes or more.	Interrupted after rise of 15 mg. % or less.	Interrupted in spite of normal rise.	Total.
5	11	6	2	24

COMMENT

The insulin-hypersensitivity of pellagrins cannot be related to the nutritional management of the patients at the time of the testing. Since the beginning of the insulin era it is well known that abundant ingestion of carbohydrates increases the insulin-sensitivity. This fact became evident in the early animal experiments for insulin assay. In human beings it was later confirmed by FALTA *et al.* (1933). The pellagrous hypersensitivity, however, is beyond the range of variations brought about by dietary changes and not influenced by them; it was invariably present in nutritional conditions, in which healthy humans showed normal sensibility.

Approximately at the time of our earlier publications MCINTYRE and BURKE (1937, 1939) reported on the insulin tolerance of the albino rat in vitamin B deficiency; the authors' experimental results are in full conformity with our clinical observations.

The normal albino rat is so highly resistant to insulin as to withstand daily injections of 200 units per kg. for 5 days without discernible effects—an amount corresponding to 12,000 units daily for a human being of 60 kg. weight.

Placed upon a diet containing no thiamin, and only small amounts of the remaining B- factors, the rats, after developing weight losses of 8 to 15 per cent., were rendered comatose and in many instances killed, by daily doses of only 20 units per kg., *i.e.* one tenth of the insulin dose previously tolerated. The enhanced effect of insulin cannot be explained by malnutrition, as control animals deprived of all food showed no marked sensitivity to insulin in 20 units per kg. daily doses, until they had lost 25 per cent. or more in body weight. Rats allowed to develop weight losses of 17 to 20 per cent. on vitamin-B free diet, supplemented with ample supplies of vitamin-B2 complex were found to tolerate easily daily doses of 20 units of insulin per kg.

The arguments suggesting adrenal damage as the possible cause of pellagrous insulin hypersensitivity were set out in the introduction to the present paper. Although some of VERZÁR's interpretations supporting this conjecture

are now controversial, the conception as a whole can be maintained In this respect the abrupt appearance of serious cerebral manifestations of hypoglycaemia not preceded by the customary phenomena of autonomic origin is a notable fact As shown by CANNON *et al* (1924) and by HOUSSAY *et al* (1924), the autonomic disturbances of hypoglycaemia are induced by the release of epinephrine from the suprarenal glands

On principle, insulin-hypersensitivity can be related to the following factors (modified after SOSKINE and LEVINE, 1946)

(1) Inability of the liver to maintain a constant blood-sugar level in response to hypoglycaemia

(2) Deficiency of hormonal insulin-antagonists

(*a*) Anterior pituitary hormones
(*b*) Cortico-adrenal hormones
(*c*) Thyroid hormone

(3) Delayed destruction of insulin in the body

Evidence of pituitary or thyroid insufficiency as factors of pellagrous insulin-hypersensitivity is not available

In leucin deficiency, hypertrophy of the hypophysis was noted (MANN *et al*, 1945), otherwise, there are no convincing reports of pituitary damage in pellagra and experimental B- avitaminoses, especially canine black-tongue, in fact, in most investigations on nutritional deficiency, not much attention was paid to the pituitary body

Information on thyroid damage in B- avitaminoses is also inconsistent In pantothenic acid deficiency, MORGAN and SIMMS (1940a) found thyroid impairment, interpreted as signs of diminished function, in grey rats, but not in white rats and other experimental animals In pellagrins, SUSMAN (1930) noted proliferation of the thyroid epithelium, fibrosis and pigmentation as a constant feature The basal metabolism of pellagrins, however, is within the normal range and the cholesterin content of the blood serum low in contrast to the high levels in myxoedema (MAINZER, 1940b) The presumption of hypothyroidism in pellagra, therefore, has no factual foundation, and the description of a subthyroid form of the disease by SHELLEY (1930) is based on rather superficial analogies

Nothing is known concerning delayed insulin destruction as a possible factor of hypersensitivity in pellagra

Since our earlier papers the outstanding importance of liver impairment in pellagra has been stressed by numerous experimental investigations on animals and clinical observations in human beings Hence it remains to review its possible influence on the insulin-hypersensitivity of pellagrins with reference to

(1) The experimental and clinical findings of nutritional liver damage related to pellagra

(2) The rôle of liver impairment in the disturbances of the blood-sugar regulation

The Italian workers on pellagra of the nineteenth century had already described liver changes, especially fatty liver, but also necrosis, cirrhosis and pigmentation as a common occurrence in pellagrins (HARRIS, 1918) More recently, atrophic cirrhosis and pigmentation was noted by WILSON (1914), fatty degeneration by BIGLAND (1920), and CRUCHFIELD (1928), fibrosis, fatty infiltration and focal degeneration by SUSMAN (1930)

In an extensive observation series from SYDENSTRICKER'S (1937) clinic (440 cases) liver damage, fatty infiltration or other impairment was found in 92 per cent.

In canine black-tongue disease the counterpart of human pellagra, SEBRELL (1929) reported on fatty infiltration of liver (and kidney). There is no need to stress the point by further references, since in fact, all investigators who paid attention to the question have reported the same findings. Not the facts, but their interpretation, was controversial. Indeed, the liver damage was mostly interpreted as due to intercurrent disease infection, worm infestation or final starvation. So, even most of the newer monographs do not comment on the subject (BICKNELL and PRESCOTT 1946), or in few words (TAYLOR and CAYER, 1947). Only HARRIS (1941) treats the matter adequately.

It was only after unravelling by animal experiments the nutritional factors effective in hepatic damage, that GILLMAN and GILLMAN (1945a) undertook a systematic study in pellagrins by liver biopsy. In the pellagra attack they regularly found *fatty infiltration*, fine or coarse, corresponding to its intensity which receded more or less completely when treated with hog stomach powder. When treated with synthetic B-vitamins (or liver extract) the fatty infiltration did not disappear in spite of the cure of other pellagra manifestations, or did so only slowly. In adult pellagrins, incompletely cured or after repeated relapses, haemochromatosis (deposition of iron containing pigment) and finally fibrosis developed, producing in extreme cases pigment cirrhosis. This final stage was observed in 12.5 per cent. of a series of 120 pellagrins.

As stressed above, the nutritional conception of liver disease in pellagrins is based on the results of animal experiments. Out of an overwhelming number of investigations, sometimes controversial, the fact seems to emerge that nutritional deficiency may induce two kinds of liver damage: fatty infiltration will develop in deficiency of lipotropic factors.

The designation "lipotropic" was coined by BEST *et al.* (1935). Lipotropic action was found for cholin and its precursors as well as pyridoxin (ENGEL, 1942), cystin, methionin and inositol (GAVIN and MCHENRY 1941). The lipotropic factors are effective against fatty infiltration with triglycerides ("fatty fat-liver"), as well as with cholesterin-esters (cholesterin-fat-liver) although to a higher degree in the former case than the latter (BEST and RIDOUT, 1933, 1938): they are active against very different influences favourable to fatty infiltration, *e.g.*, excessive cholesterin feeding, starvation, anterior pituitary hormone and liver poisons. Cholin and methionin may be substituted reciprocally since both substances make available the methyl group necessary for the biological synthesis of the other one. This process of transmethylation has been shown to take place in human beings as well as in different laboratory animals (DU VIGNEAUD, 1942, 1943).

On the other hand, deficiency of sulphur-containing aminoacids, *e.g.*, methionin and cystin, may induce *focal hepatic necrosis* and haemorrhage (GYÖRGY and GOLDBLATT 1939, 1942). Both fatty infiltration and necrosis may be followed by fibrosis, a development which is more or less prevented by methionin or cholin feeding (DAFT *et al.*, 1942). If not fatal, massive hepatic necrosis will finally produce cirrhosis with coarse nodular hyperplasia ("post necrotic scarring") (GLYNN and HIMSWORTH, 1944a, 1944b). The fatty infiltration may progress to diffuse hepatic fibrosis (classical portal cirrhosis). Therefore the two final stages of nutritional liver damage are different aetiologically as well as morphologically.

The aetiological theory of the pellagra liver is founded on these experimental findings. Cholin and pyridoxin, two factors of the vitamin B group, as well as cystin and methionin, two essential amino-acids and monomers of high value protein, are altogether deficient in nutritional conditions enhancing pellagra. Thus, with reference to liver impairment, two theories of pellagra aetiology, once strictly opposed, are coming together.

WILSON'S (1921) theory of high value protein deficiency, and the theory of a specific vitamin deficiency put forward by FUNK (1911) and SANDWITH (1913) and substantiated by GOLDBERGER and associates (1915) The two theories have also been amalgamated with respect to nicotinic acid, since the essential amino-acid tryptophan has been found to be its biological precursor (ROSEN *et al*, 1946) Likewise, MELLANBY'S (1934) idea of a "toxamin," a toxic factor present in maize and effective only in simultaneous nutritional deficiency, has fused with these theories, since, as shown by WOOLLEY (1946), maize contains an antivitamin, a pellagrogenic substance increasing the minimal need for nicotinic acid

It is a well known fact that liver damage may induce disturbances of carbohydrate metabolism in human disease (liver cirrhosis, acute yellow atrophy) as well as in animals (coccidiosis of rabbits, liver poisons) Decrease of the blood-sugar was noted in phosphorus and chloroform poisoning (MANN and MAGATH, 1924, MANN, 1927), but this is not a regular occurrence The same observation was made in diffuse human liver disease (CONN *et al*, 1938, LICHTWITZ, 1942, and others)

Numerous investigations of sugar tolerance, using peroral or intravenous ingestion of different sugars, were carried out in these conditions, otherwise clinical research on the subject is scanty Experiments on insulin sensitivity in human liver disease or liver poisoned animals are not reported SHAY *et al* (1931) studied metabolism of galactose in human beings, in some experiments also under the influence of insulin, similar studies were made by ROE and SCHWARTSMAN (1932) in humans and rabbits, but these investigations, concerned primarily with galactose metabolism, dealt with insulin only incidentally, furthermore, pertinent facts were not observed BUERGER (1930) tried to make use of the initial hyperglycaemia induced by some earlier brands of insulin (especially the Wellcome brand) as a liver function test This hyperglycaemia was brought about by some impurity of the insulin preparations (later eliminated), and since the author made estimations of the blood-sugar only within the first 30 minutes after injection, he missed the opportunity for an investigation of insulin-sensitivity in liver disease

MANN and MAGATH (1923) found the insulin effect on the blood-sugar more tardy after partial or total liver extirpation than before, it results from their experiments, that the *influence of the liver on the insulin-blood-sugar-curve* is manifest merely in the compensatory rise to the initial level

As reported by NOBEL and MACLEOD (1923a, 1923b) and by MCCORMICK and MACLEOD (1925), the extent of insulin hypoglycaemia and the subsequent rise of the blood-sugar are closely related to the glycogen stores of the liver Nevertheless, depletion of liver glycogen cannot account for the persistence of insulin hypoglycaemia in pellagrins, since the insulin-hypersensitivity remains unchanged for many weeks in spite of abundant nutrition and gain of weight The glycogen stores—it must be assumed—even if insufficient in the beginning, would have been repleted

If not the unavailability of liver glycogen, the insufficiency of neoglycogenesis from non-carbohydrate sources, must produce the tardy and deficient sugar output to the blood Neoglycogenesis is claimed to be inhibited by lack of cortical hormone (JENSEN and GRATTAN, 1940, GRATTAN *et al*, 1941), but liver damage may also interfere Actually, the persistence of insulin-hypersensitivity in pellagrins in spite of clinical cure is well in line with the persistence of fatty infiltration of the liver, despite cure, as described by GILLMAN and

GILLMAN (1945b), and their statement that nicotinic acid has no curative effect on this manifestation. Investigations with cholin were, apparently not made by the South African authors nor by ourselves at the time of our experiments, neither cholin nor pantothenic acid were commercially available Therefore it remains open to question whether the deficiency of neoglycogenesis is due to liver damage or to cortico-adrenal impairment. Animal experiments on insulin-sensitivity in canine black-tongue, in pantothenic acid deficiency and in cholin and protein deficiency will probably give the definite answer

SUMMARY

Pellagrins exhibit with notable regularity hypersensitivity to insulin. In two-thirds to three-quarters of patients small doses of insulin (5 units subcutaneously) induce a fall of the fasting blood-sugar largely exceeding the effect in normal humans and not infrequently progressing to levels between 10 and 20 mg per cent. In a quarter of the experiments severe cerebral manifestations of hypoglycaemia would appear abruptly often not preceded by autonomic phenomena. In most cases the compensatory rise of the blood-sugar to the initial level is tardy and incomplete it may be entirely absent.

Two organs implicated (with others) in the regulation of carbohydrate metabolism, the adrenal glands and the liver show severe anatomical damage in most pellagrins. The adrenals present enlargement, lipoid depletion, necrosis and haemorrhage in the liver fatty infiltration, focal necrosis, fibrosis and haemochromatosis are reported as regular findings.

The liver impairment is probably induced by deficiencies of cholin and high value protein, the adrenal damage by pantothenic acid deficiency

Some arguments are in favour of the adrenal damage as a factor effective in the insulin hypersensitivity of pellagrins there are numerous and important clinical analogies between Addison's disease and pellagra, an apparent biochemical identity of the disturbances of intestinal absorption in both diseases, and the frequent absence during the hypoglycaemia in pellagrins of autonomic symptoms, produced by epinephrine output.

On the other hand the possible rôle of pellagrous liver damage in bringing about the phenomenon of insulin hypersensitivity is strongly suggested by investigations of the last few years.

The insulin hypersensitivity cannot be related to depletion of the liver glycogen stores, since it will persist without exception after restoring the nutritional state hence it must be due to insufficient neoglycogenesis from non carbohydrate sources.

Since cortical hormones are implicated in neoglycogenesis, and the liver is the site of this biochemical transformation, the present observations do not solve the question. This is expected to be finally decided by animal experiments on insulin-sensitivity in canine black tongue deficiencies of cholin and high value protein and pantothenic acid deficiency

REFERENCES

ASCHOFF, L (1933) *Munch med Wschr*, 1, 302
BEST, C H, HUNTSMAN, M E & RIDOUT, J H (1935) *Nature*, 135, 21
——— & RIDOUT, J H (1933) *J Physiol*, 78, 415
——— & ——— (1936) *Ibid*, 86, 343
BICKNELL, F & PRESCOTT, F (1946) *The Vitamins in Medicine* 2nd Ed London Heineman
BIGLAND, D A (1920) *Lancet*, 1, 947
BOLLER, R, DEPISCH, P & FALTA, W (1933) *Grundlagen der Diabetestherapie*, Wien, Urban
BUERGER, M (1930) *Klin Wschr*, 9, 104
BURKE, J C & MCINTYRE, A R (1939) *J Pharmacol*, 67, 142
CANNON, W B, MCIVER, M A & BLISS, S W (1924) *Amer J Physiol*, 69, 46
CONN, J W, NEWBURGH, L H, JOHNSTON, M W & SHELDON, J M (1938) *Arch intern Med*, 62, 765
CRUTCHFIELD, E D (1928) *Arch Derm Syph*, 17, 650
DAFT, F S & SEBRELL, W H (1939) *Publ Hlth Rep*, 54, 2247
———, ——— & LILLIE, R D (1942) *Proc Soc exp Biol*, 50, 1
DUVIGNEAUD, V (1942) *Harvey Lect*, 38, 1
ENGEL, R W (1942) *J Nutr*, 24, 175
FERREBEE, J W (1940) *J biol Chem*, 136, 719
FLETCHER, A A & CAMPBELL, W R (1922) *J metab Res*, 2, 637
FINDLAY, G M (1928) *J Path Bact*, 31, 353
FINOTTI, R & TEDESCHI, E (1902) *Rif med*, 2, 230
FRASER, R, ALBRIGHT, F & SMITH, P H (1941) *J clin Endocrin*, 1, 300
FROBOESE, C (1934) *Zbl Path*, 60, Erg -Heft, 124 and 226
——— & THOMA, E (1933) *Z klin Med*, 124, 478
FUNK, C (1911) *J Physiol*, 43, 395
——— (1919) *Ibid*, 63, 247
GAVIN, G & MCHENRY, E W (1941) *J Biol Chem*, 139, 485
GILLMAN, J & GILLMAN, TH (1945a) *Arch Path*, 40, 239
GILLMAN, TH & GILLMAN, J (1945b) *Arch intern Med*, 76, 63
GLYNN, L E & HIMSWORTH, H P (1944a) *J Path Bact* 56, 297
——— & ——— (1944b) *Clin Sci*, 5, 93
GOLDBERGER, J & WHEELER, G A (1915) *Publ Hlth Rep*, 48, 3336
GRATTAN, J F, JENSEN, H & INGLE, D (1941) *Amer J Physiol*, 134, 8
GYÖRGY, P & GOLDBLATT, H (1939) *J exp Med*, 70, 185
——— & ——— (1942) *Ibid*, 75, 355
HARRIS, H F (1918) *Pellagra* New York Macmillan
HARRIS, S (1941) *Pellagra* St Louis Mosby
HERZENBERG, H (1935) *Beitr path Anat*, 96, 97
HOUSSAY, B A, LEWIS, J T & MOLINELLI, E A (1924) *C R Soc Biol*, 91, 1011
JENSEN, H & GRATTAN, J F (1940) *Amer J Physiol*, 128, 270
LICHTWITZ, L (1942) *Functional Pathology*, 534 New York Heinemann
LUCKE, H (1933) *Z ges exp Med*, 87, 103
LYMAN, R S, NICHOLS, E & MCCANN, W S (1923) *J Pharm Exp Ther*, 21, 377
MACLEOD, J J R & ORR, M D (1924) *J Lab Clin Med*, 9, 591
MCCARRISON, R (1921) *Studies on Deficiency Disease* Oxford Henry Frowde and Hodder & Stoughton
MCCORMICK, N A & MACLEOD, J J R (1925) *Proc Roy Soc* B 98, 1
MCINTYRE, A R & BURKE, J C (1937) *Amer J Physiol*, 119, 364
MCQUEENY, A J, ASHBURN, L L, DAFT, F S & FAULKNER, R (1947) *Endocrinology*, 41, 441
MAINZER, F (1939) *Act Med Scand*, 100, 208
——— (1940) Unpublished data
——— & KRAUSE, M (1940) *Ibid*, 104, 21
MANN, C F (1927) *Medicine*, 6, 419

posterior root ganglia were demonstrated as well as grosser lesions of Clarke's column in which scarcely a cell was normal. This confirmed an early observation of WILSON in 1914. The case to be described below illustrates the spino-cerebellar element, and, interestingly enough, when first examined by a young physician from England familiar with neurological technique, but unfamiliar with these syndromes, was diagnosed as a cerebellar abiotrophy.

CASE REPORT

C.A.S., male Chinese, aged 68 was admitted to the General Hospital, Singapore, on 4th December 1948 for numbness of the extremities, unsteadiness in walking and gradual blindness of the left eye of 6 weeks duration.

The symptoms appeared few weeks after he had lost his job as hand-carter. Not having much money he slept on covered pavements and ate very little. Numbness appeared over the hands and feet and spread centrally to involve the arms and thighs so that on admission the distal parts of the extremities were more numb than the proximal. Together with this there was an inability to use his fingers which progressed so that his friends had to feed him. Walking became more and more difficult, until eventually he was bedridden.

On examination, the patient was wasted old man with dry rough skin, mentally apathetic. The left eye was blind from leucoma, while the right was normal. There was no nystagmus. Angular stomatitis and dermatitis of the scrotum were present. The tongue edges were smooth. No muscular weakness was found in the upper or lower limbs but there was gross inco-ordination intention tremor and dysmetria on performing the finger-nose and heel-knee tests. Dysdiadochokinesia was present. The gait was ataxic and walking impossible without assistance. Rombergism was not present. There was very slight impairment of sensation to touch and pinprick over the hands and feet, but this was not sufficient to account for the ataxia. Neither the vibration nor the position sense was affected. The calf muscles were tender on deep pressure and the testicular sensation was present. All the tendon reflexes were abolished. The abdominal reflexes were normal and the plantar responses flexor. Sphincter control was maintained.

The cerebrospinal fluid was normal. The Kahn test was negativ in both cerebrospinal fluid and blood. The gastric test meal showed an absence of free hydrochloric acid. The red cell count was 3 5 million and the haemoglobin was 10·9 grammes.

He was put on a liberal diet, with supplementary milk and eggs, and given a course of nicotinic acid and riboflavin. By the tenth day of treatment he was alert, talkative and smiling. The tongue had returned to normal and the scrotal dermatitis was clearing up. The inco-ordination and ataxia were still present, but he could walk unaided. Examined 3 months later his general appearance was good and there was no evidence of ataxia. The red cell count was 4 5 million and the haemoglobin was 12·4 grammes. Free hydrochloric acid was still absent in all the specimens of the gastric test meal.

REFERENCES.

DENNY BROWN, D (1947). *Medicine* **26**, 41
LANDOR, J V C & PALLISTER, R. A. (1935) *Trans. R. Soc. trop. Med. Hyg.* **29** 121
PALLISTER, R. A. (1940) *Ibid* **34** 203.
RANSOME, G A. (1944). *Brit. med. J.* **2** 637
SPILLANE, J D (1947). *Nutritional Disorders of the Nervous System* (Monograph). Edinburgh E. & S. Livingstone Ltd
SANDS, H S. (1944) *Brit. med. J.* **2** 103 140
WILSON S A. K. (1914). *Proc. R. soc. Med.*, **7** 31 Sect. of Neurol.

Before treatment.

Three months after treatment

To face page 341

Transactions of the Royal Society of
Tropical Medicine and Hygiene.
Vol. 43. No. 3. November, 1949.

OBITUARY.

Sir Herbert J. Read, G.C.M.G., C.B.

In the death of Sir Herbert Read the Society has lost one of its distinguished Honorary Fellows, one who was elected as long ago as 1921.

As Assistant Private Secretary to Mr. Joseph Chamberlain in 1895 Mr. Read became intimately associated with the Colonial Office and got to know about the unhealthy state of the African Colonies, conditions which were very disturbing to Mr. Chamberlain as Colonial Secretary. At this time Dr. Manson had come home from China and the idea of tropical teaching was discussed, the result being the foundation of the London School of Tropical Medicine and Hygiene. Read, working behind the scenes, had much to do with this and it is right that the part he played in it should be more widely recognized than it has been. Then we have the *Sleeping Sickness Bulletin*, the *Tropical Diseases Bulletin* and the *Bulletin of Hygiene*, all of the greatest value to tropical workers. Read had much to do with the foundation of these and sat on their management committees. He also joined in 1898 the Committee of Management of the Seamen's Hospital Society, the body associated with the tropical school and the source of tropical cases, and was a Vice-President of that Society at the time of his death.

In view of his great services to tropical medicine our Society elected him an Honorary Fellow in 1921, and later on he was very helpful and active in the establishment of Manson House, our Headquarters, opened by the then Prince of Wales on 17th March, 1932. Read was also interested in the Liverpool School of Tropical Medicine and was a Vice-President there as well. Outside tropical medicine he had many other activities, for example, he was Governor of Mauritius 1924 to 1930, and when he retired was chairman of the executive committee of the Royal College of Science and Technology.

Sir Herbert was of a modest and retiring nature, charming to meet at all times and a general favourite. He was created C.M.G. in 1907 and C.B. in 1914, and promoted K.C.M.G. in 1918 and G.C.M.G. in 1935.

G. C. L.

TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE
Vol 43 No 3 November, 1949

CORRESPONDENCE.

"DDT SPRAYING IN BRAZIL"

To the Editor, TRANSACTIONS *of the Royal Society of Tropical Medicine and Hygiene*

SIR,

In a paper entitled "Tropical Diseases in Brazil," presented by Professor MALAMOS at the ordinary meeting held by the Royal Society of Tropical Medicine and Hygiene, and later published in the TRANSACTIONS, **43**, 1, there is a reference to the National Malaria Service which is not exact, and which we wish to explain

On page 18 of his paper, Professor MALAMOS states

"Every year DDT is employed in the country on a larger scale DDT spraying has been carried out only from airplanes (helicopters) but, as I was told, the large extent of waterways and distribution of the breeding places prevent favourable results In some southern districts only, this method gave good results In the Amazon valley, spraying from the air would be a waste of work and money The method of choice is the spraying of all the houses in an endemic area"

Professor MALAMOS has obviously made a mistake The spraying of DDT from helicopter by the National Malaria Service was carried out only in limited areas of the State of Santa Catarina, and in an experimental way, against anophelines of the sub-genus *Kerteszia*, vectors of malaria in south of Brazil In all other States of Brazil DDT is used inside the houses, and until July of the present year, National Malaria Service had already treated with DDT 2,206,261 houses, which number will very soon reach 3,000,000 Even in the area where malaria is transmitted by *Kerteszia* (States of Parana, Santa Catarina and Rio Grande do Sul), DDT is now applied inside the houses with satisfactory results Experimental spraying with DDT by helicopter in the State of Santa Catarina was without success, the method proved to be very expensive In *Revista Brasileira de Malariologia*, **1**, 2, are published the results of first experiments made with DDT spraying by helicopter Further experiments gave more definite results, and that method was found impracticable

No doubt language difficulties are responsible for the misunderstanding, as it did not occur to those who are responsible for malaria control in Brazil to apply DDT spraying by helicopter all over the great extent of our country

I am, etc

MARIO PINOTTI,
Diretor do Serviço Nacional de Malária

Rio de Janeiro,
30*th September*, 1949

TROPICAL DISEASES IN BRAZIL

SIR,

I have read with great interest in the TRANSACTIONS, 43 11 the lecture given by Professor B. MALAMOS to the members of the Society It seems to me that the comments made at the meeting especially those of Sir GEORGE MCROBERT may have produced a false impression as regards Brazil. To say that to the man in the street, Brazil is known as the "home of coffee and Carmen Miranda" is just as relevant as to affirm that to the man in the street in Rio de Janeiro England is the home of whisky and the players of the Southampton F C. To our cultural university graduates, however England suggests SHAKESPEARE and BERNARD SHAW PITT and CHURCHILL NEWTON and RUTHERFORD JOHN LOCKE and HERBERT SPENCER RONALD ROSS and FLEMING.

As a Fellow of the Society I should feel extremely grateful if you would allow me to correct some mistaken notions which may be spread by Professor MALAMOS lecture.

For this purpose, I beg leave to forward (under separate cover) some of our medical publications in the hope that you will exhibit them in some place available to the Fellows, and later house them in the Society's library I have also included some views of Brazilian towns, among them our city of Belo Horizonte, which is 600 km. distant from the coast. Here there is a young but flourishing university in which I have the honour of occupying the Chair of Tropical Medicine. From the photographs you will see that, in spite of its scant 50 years of existence, Belo Horizonte offers all the amenities of a European town and a tranquility which, in Europe, is not easily obtainable. Its material and cultural progress has been extremely rapid, and the University of Minas Gerais, with a roll of 2,000 students, has Faculties of Medicine Pharmacy Odontology Chemistry Law Philosophy Engineering Architecture, Economic Sciences, Agronomy and Veterinary Medicine. There is also a considerable number of institutes for providing secondary education. It is hardly necessary to add that the larger cities of Rio de Janeiro S. Paulo, Porto Alegre Salvador and Recife offer greater and more extensive cultural opportunities.

This is Brazil of today wonderful in all its aspects, crossed from north to south and from east to west by planes which put every part of its territory into contact with the rest of the world. There would seem, therefore, to be no grounds for the fears expressed by Sir GEORGE when he says "*We must make sure that they are* (international statesmen) *advised not to submit unsalted and unseasoned innocents to the dire hazards which Professor* MALAMOS *has revealed to us tonight*"

It is true that beyond this modern Brazil there lies the "vast green vacuum" of our forests in Central Brazil and the Amazon Valley There undoubtedly one can meet with surprises but they are not dissimilar to those to be encountered in the English colonial jungles, with this important difference

that our forests constitute an inalienable part of our undivided country and, as such, will be maintained as a guarantee for our country's further greatness

I shall feel sincerely obliged if you bring to the notice of those who heard Professor MALAMOS' lecture this letter and the photographs and literature which I am forwarding by surface mail

I am, etc ,

OSCAR VERSIANI CALDEIRA

2344, Rua Timbiras,
Belo Horizonte, M G ,
Brazil

6th October, 1949

SIR GEORGE MCROBERT has replied to the above letter as follows

SIR,

Members of our Society who devoted three successive meetings last session to problems of South America—Brazil, Uruguay and Venezuela—are not likely to form " a false impression " of the homeland of Professor CALDEIRA from hearing or reading the interesting and informative lecture given by Professor MALAMOS

Professor MALAMOS was careful to give a friendly and balanced account of the Government of Brazil, of its administrative structure and of the serious problems which it has to face—problems which, at the request of Brazilian representatives, U N E S C O investigated The lecturer mentioned the poverty of some of the states and the prevalence of serious disease In the subsequent discussion I drew attention to the possible danger of international statesmen looking to such tropical vacua as likely resettlement areas for displaced persons never previously exposed to malaria and yellow fever

Dr MALAMOS made special mention of the great universities and medical schools of Brazil In this Society we have all been aware for many years of the fine work and international repute of the institutes at Rio de Janeiro and Sao Paulo and of the outstanding Brazilian contributions to medicine, general science and jurisprudence

That " the man in the street " here to whom I referred knows little of Brazil except through its export contributions to his cinema entertainment and breakfast table, is quite true By " the man in the street " we do not mean " cultured university graduates " but the average citizen who votes in elections, pays the taxes and determines the nature of the government Those of us who have lived for many years in India are acutely aware of the ignorance of " the man in the street " in England about major members of the British Commonwealth

Professor CALDERIA may rest assured that his country is held in great regard by those who understand its problems, sympathize with its difficulties and admire the efforts to overcome them

NEW FELLOWS

At the meeting of the Society held at Manson House on 17th November 1949 the following 22 candidates were elected Fellows of the Society —

ASHTON MARY A., M.B., CH.B. (EDIN.), D.T.M. & H. (EDIN.), Scotland
BRADY F J M.D. (MICHIGAN), U.S.A.
CHOUDHURI, R. K. D B.SC. (CAL.) M.B. (CAL.), India.
CULLUMBINE, HARRY M.D CH.B M.SC., Professor of Physiology Ceylon.
DWORK, KERMIT G M.D. (NEW YORK), Cert. in Tropical Medicine (Washington) U.S.A.
EGERTON, MARY E., M.B., B.S. (LOND.) M.R.C.O.G., Sierra Leone.
FLINDT M. L. H., M.B., B.S. (LOND.), M.R.C.S. (ENG.) L.R.C.P (LOND.) Sarawak.
GEORGE, BRYCHAN, M.R.C.S. (ENG.), L.R.C.P (LOND.) Sierra Leone
GRAY H H., M.D. (TULANE), U.S.A.
GUPTA, PREM. NATH B.SC. (PUNJAB).
HOBSON A. C. S M.C., M.B. B.S. (LOND.) M.R.C.P (LOND.) Major R.A.M.C., Sierra Leone
JONES C. R., B.SC., M.B., B.CH. (WALES) British Guiana.
KONG, HOI KIT M.B., B.S. (HONGKONG), D.T.M. & H. (ENG.) Hongkong.
KOWALDO, L., M.B., B.S. (MELB.), Australia.
MACKENZIE, C. R., M.B B.CH., D.P.H. (W'RAND), British Guiana.
POMALES-LEBRON, AMERICO M.S. (Puerto Rico), PH.D. (Michigan), Puerto Rico.
REID, J D M.B., CH.B., D.P.H. (EDIN.) D.T.M., D.C.P (LOND.) Sierra Leone
ROCHESTER, W K. M.B., B.S., D.T.M. & H. Sierra Leone
SALAWU F., L.S.M. (NIGERIA), Nigeria.
STEWART A. F H., M.R.C.S. (ENG.), L.R.C.P (LOND.) D.P.H. (LOND.), D.T.M. & H (ENG.) Trinidad.
TECK, LEE, M.B. (CHINA), D.T.M. & H. (ENG.), China.
WELLER, THOMAS H M.D (HARVARD) Ass. Prof Tropical Public Health, U.S.A.

LIBRARY NOTICE

NEW BOOKS RECEIVED

Golden Jubilee World Tribute to DR. SIDNEY V HAAS.

Amoebiasis By G P G SOBHY BEY Cairo Fouad I University Press.

A Manual of Practical Tropical Sanitation. By J BALFOUR KIRK. London Baillière Tindall & Cox.

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Papers should, if possible, be typewritten; they should be concisely written with subject matter logically arranged and sub-divided; with references and abbreviations in the form described below; and with indications of the position, in the text, of illustrations, tables, maps, etc.

Titles should be as brief as consistent with clarity; and in many cases the value of a paper is enhanced by a short **summary** at the end.

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In the text, the date of publication, in brackets, *should follow the name of the author quoted* thus :—

"To MANSON (1878) is due this epoch-making discovery."

At the end of the paper a *list of References should be arranged in alphabetical order of authors'* surnames, and details given in the following order :—(1) Surname of author ; (2) Initials of author ; (3) Year of publication, in brackets ; (4) Title of article, avoiding arbitrary capitals. (The title of the article is sometimes omitted ; but each list of references should in this respect be consistent throughout—giving all titles or omitting all). (5) Title of journal ; (6) Volume number ; (7) Page number ; *e.g.* :—MANSON, P. (1878). On the development of *Filaria sanguinis hominis* and on the mosquito considered as a nurse. *J. Linn. Soc. (Zool.),* 14, 304.

In the case of a reference to a book : (1), (2) and (3) as above ; (4) Title of book ; (5) Edition and/or volume, if more than one ; (6) Page number ; (7) Town of publication ; (8) Publisher's name, *e.g.* :—MANSON, P. (1898). *Tropical Diseases,* 1st Ed., 447. London : Cassell & Co., Ltd.

Reference to an Annual Report : SWAZILAND (1937). *Annual Medical & Sanitary Report 1936,* p. 16.

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The abbreviations used are those shown in the **World List of Scientific Periodicals, 1934,** which conforms to the rules of the *International Code of Abbreviations for Titles of Periodicals,* Paris, 1930. In general, *nouns have capital, adjectives small, initial letters* ; articles, conjunctions and prepositions are omitted ; the place of imprint is added only when uncertainty might arise, *e.g.* :

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Ann. trop. Med. Parasit.	*C. R. Acad. Sci., Johannesburg.*	*Ned. Tijdschr. Geneesk.*
Arch. Schiffs- u. Tropenhyg.	*Dtsch. med. Wschr.*	*Trans. R. Soc. trop. Med. Hyg.*
Bull. Soc. Path. exot.	*Indian med. Gaz.*	*Z. Hyg. InfektKr.*

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centigramme, cg.	kilometre, km.	millimetre, mm.
centimetre, cm.	micron, μ.	ounce, oz.
cubic centimetre, c.c.	microgramme, μg.	pound, lb.
cubic millimetre, c.mm.	milligramme, mg.	
kilogramme, kg.	millilitre, ml.	

In order to avoid dangerous error in dosage, "gram" and "gramme" are printed in full.

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TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

JANUARY, 1950 VOLUME 48 No 4

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[The previous number of these Transactions, Vol 43 No 3 was published on 28th November, 1949]

TRANSACTIONS
OF THE
ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

VOL 43 NO 4 JANUARY, 1950

LABORATORY MEETING

of the Society held at

The London School of Hygiene and Tropical Medicine, Keppel Street, London, W C 1,

2 *Microfilaria melleri* in *Chameleon melleri*

3 Pre-erythrocytic schizonts of *P falciparum* of approximately 4th, 5th and 6th day stages were shown, the most mature exhibiting ripe merozoites about to invade the blood stream

Dr P C C Garnham

Exo-erythrocytic schizogony in *P pitmani*, a malaria parasite of the East African lizard

Smears from the heart and spleen of *Mabuia maculilabris* illustrated the development of unpigmented schizonts of *P pitmani* in lymphoid macrophage cells

REFERENCE GARNHAM, P C C (In the press) Blood Parasite of East African Vertebrates *Parasitology*

Dr P L Le Roux

***Onchocerca gutturosa* infestation in cattle in Wales**

(1) Hygroma attached to the *ligamentum nuchae* of an aged cow, Hertfordshire, England

It would appear that the localisation of micro-organisms, especially *Brucella abortus* in the tissues in certain localities of the bovine body is closely connected with *Onchocerca gutturosa* infestations. *Br abortus* in pure culture was isolated from the exhibited specimen. In the horse analogous lesions develop at the withers and the poll, the habitats of *O cervicalis* which is morphologically identical with *O gutturosa*. The invasion of the worm-infested tissues by micro-organisms is responsible for fistulous withers and poll-evil in equines. The importance of hygromas in animals as a source of brucellosis in man should not be overlooked at meat inspection in temperate regions and in the tropics.

(2) *Onchocerca gutturosa* in the loose areolar connective tissue on the medial surface of portions of *ligamentum nuchae* from four cows, Carmarthenshire Wales. Bursa formation, pre-hygromal stage, over the helminths is evident in two of the specimens. Congestion of the blood vessels in the tissues adjacent to the worms is well marked in the others.

Bilharziella

(3) A schistosome (*Bilharziella* sp.) egg in the caecal wall of a wild mallard (*Anas platyrhynchos* L.) shot in Regent's Park, London. The shape of the egg resembles that of *Bilharziella yokogawai* Oiso 1927 from the domesticated duck, Formosa. Schistosome dermatitis due to the invasion of man by cercariae of avian origin has been reported from parts of the British Isles.

Dr A A Sandosham

Some species of *Enterobius* from Primates.

A number of line drawings was exhibited to illustrate the distinguishing features of the parasites.

1 **Tip of tail of male *E. vermicularis* mounted as a hanging-drop preparation.**

Hall (1933) pointed out that different investigators did not agree regarding the number of caudal papillae in the male of this very common human parasite, and that everyone except Leuckart had overlooked the small pair of sessile papillae situated lateral to the most anterior pair of pedunculated papillae. Examining a large number of males it was noted that this pair of papillae which was usually difficult to see, became more obvious when the tail end was mounted as a hanging-drop preparation.

This technique has been in use by Professor Buckley and is very suited for the examination of the *en face* view of head ventral view of the tip of tail, etc. The specimens can be examined in any clearing medium and can easily be adjusted to any required angle. There is no pressure of coverglass on the specimen.

2 ***Enterobius vermicularis* from a chimpanzee (*Pan. satyrus*) in the London Zoological Gardens**

This material was obtained by Dr R. E. Rewell from a 3-year-old chimpanzee in the London Zoological Gardens and sent to Professor Buckley for examination.

E. vermicularis is a human parasite and this is the first record of it in a

different host CAMERON (1929) pointed out that, owing to its peculiar life history, the genus *Enterobius* tends to be a parasite of the individual, and in consequence each species is likely to be restricted to one kind of host BUCKLEY (1931), recording the first exception to this rule, states that " the exception is explicable on the supposition that the restriction is only one of habit, and that a physiological host parasite relationship has not become established "

The finding of *E vermicularis* (hitherto only recorded from man) in a young chimpanzee in the London Zoological Gardens, where it is handled by the staff and probably fed by children visiting the Zoo, substantiates his views

Interest to the medical profession lies in the possibility of human infection of *Enterobius* of other Primates through keeping pet monkeys or visiting Zoological Gardens Several problems in the life-history of *E vermicularis* such as hyper-infection, retro-infection, etc , remain to be solved The finding that the chimpanzee can be infected with the human *Enterobius* opens up possibilities of it being used as an experimental animal by research workers

3 The hitherto unknown male of *Enterobius anthropopitheci* (Gedoelst, 1916) from the Chimpanzee (*Pan satyrus*)

The material was collected by Dr P L LE ROUX from a chimpanzee that died in the laboratory soon after its arrival in London

GEDOELST described only the female of this species BAYLIS and DAUBNEY (1922) obtained some specimens from a black-headed lemur in India which they tentatively determined as *E anthropopitheci* Their material also consisted only of females MOORMAN (1941) records finding one female pinworm in a chimpanzee in the U S A but there appears to have been no attempt to determine the species.

4 *Enterobius* n sp from the orang-utan (*Pongo pygmaeus*)

The material was collected by me from the intestines of an orang-utan that died in the laboratory and placed at my disposal by Professor BUCKLEY

Two species of *Enterobius* have been described from the orang, namely, *E faecundus* (V Linstow, 1879) and *E simae* (MacCullum, 1921)

5 *Enterobius* n sp from the gorilla (*Gorilla gorilla*)

One male and several females were sent by Dr ANNIE PORTER from the London Zoological Gardens where they were obtained from the washings of faeces of a young gorilla after treatment.

There are no records in literature of an *Enterobius* from the gorilla

6 *Enterobius* n sp from the Chaoma baboon (*Papio comatus*) from Southern Rhodesia

This material belongs to the helminthological collection of the London School of Hygiene and Tropical Medicine and had been collected by Dr W K BLACKIE during his survey in Southern Rhodesia

This is the first record of an *Enterobius* from this host

7 *Enterobius* sp from the Guenon monkey (*Cercopithecus aethiops*) from Southern Rhodesia

This material belongs to the helminthological collection of the London

School of Hygiene and Tropical Medicine and had been collected by Dr W K BLACKIE during his survey of Southern Rhodesia.

E. bipapillatus (Gedoelst, 1916) has been recorded from an undetermined monkey from Central Africa and from *Cercopithecus sabaeus* from West Africa. The *Enterobius* from *C aethiops* differs in certain respects from *E. bipapillatus* but it is not clear if the differences are of specific significance.

8. *Enterobius* sp from the lion marmoset (*Leontocebus rosalia*) from S.E Brazil

This material belongs to the helminthological collection of the London School of Tropical Medicine and hygiene and had been collected at the London Zoological Gardens.

This is the first record of an *Enterobius* from this host. *E callithricis* (Solomon, 1933) has been described from another marmoset (*Callithrix jacchus*) from South America but this species seems to be different.

Miss Sheila Willmott (introduced by Professor J J C Buckley)

A new species of *Paramphistomum* from Scottish cattle

It has been assumed that the only member of the family Paramphistomidae which occurs in this country is *Paramphistomum cervi*. On examining a number of specimens collected from the rumens of Scottish cattle killed on the Isle of Mull and in the Municipal Abattoir Glasgow it became apparent that two species occur One is believed to be *P cervi* and the other to be a new species

Hand specimens, and a median sagittal section of each species, were shown.

DEPARTMENT OF MEDICAL ENTOMOLOGY

Dr D S Bertram.

Cotton rat filariasis factors affecting the intensity of infection in the vector *Liponyssus bacoti*.

Graphs were presented to show that the infectivity of a cotton rat is more closely associated with the age of its infection than with the density of microfilariae in its peripheral blood.

Dr J R Busvine

A film on the Mosquito eradication campaigns in Sardinia and Cyprus

Two short 16 mm. silent films were shown. The first showed some of the countryside of Sardinia, followed by sections devoted to different aspects of the eradication campaign. Thus (1) Entomological survey (2) Anti-adult programme of house spraying with DDT (3) Anti larval treatments by DDT applied by hand sprayers (4) Checking the work.

The film on Cyprus was in colour and showed typical features of the country and its inhabitants. Various aspects of the anopheline eradication work were illustrated.

Miss C M Harrison

DDT-resistant flies

The failure of DDT to control house flies was first reported in 1947 and related to flies in Sweden Since then there have been reports of DDT-resistant flies from Italy, Denmark and America

Flies of two strains, one non-resistant, and the other DDT-resistant (derived by selection from a partially resistant strain of Italian origin), were exposed to a glass surface treated with DDT at the rate of 12 mg per sq ft The demonstration began at 7 45 p m By 9 30 p m all flies of the non-resistant strain were lying on their backs incapable of righting themselves, whereas all flies of the DDT-resistant strain were unaffected

A series of graphs demonstrated the stages in the production of a pyrethrin-resistant strain of house flies by selection over five generations This pyrethrin-resistant strain was shown to be more resistant to DDT than the normal strain

LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Department of Tropical Medicine

1 Method of estimating oxygen carried by the blood

Modification of Haldane method of blood gas analysis using new micro technique adapted for Warburg apparatus

2 Micro-anatomy of the liver

A full account of the vascular micro-anatomy is given by ANDREWS et al (1949) The demonstration consisted of specimens used for this paper and included Neoprene and Hycar vascular casts, coloured gelatine and indian ink injections, and a section of liver which had been perfused with both the diazo salt α-amino anthroquinone and 2-naphthol-6-sulphonic acid A diagram of the circulation was included

REFERENCE *Ann trop Med Parasit* (in the press)

Department of Entomology and Parasitology

Dr W E Kershaw

The treatment of experimental filariasis with MSb (Friedheim)

The pentavalent antimonial drug sodium p-melaminylphenylstibonate (MSb) (prepared by Dr E A H FRIEDHEIM) in its polymerized form is precipitated after injection into the peritoneal cavity, and is eliminated slowly, thus maintaining a sufficiently high blood level to produce prophylactic activity

It has very marked prophylactic activity in experimental trypanosome infections

In experimental filariasis in the cotton rat, one injection of 250 mg per kilo body weight was originally shown to be an efficient prophylactic for at least 3 weeks, and this prophylactic action has subsequently been found to persist for 6 months

The demonstration showed the method of assessing the action of drugs in established experimental filariasis used at the Liverpool School of Tropical Medicine, and the results of some preliminary experiments on the effect of MSb on these infections.

The action of drugs effective in the treatment of filariasis may be predominantly on the microfilariae, as is the case with hetrazan, or upon the adults, as occurs with the organic antimonial derivatives.

The method of assessing these two separate effects is dependent upon knowledge of the early stages of the evolution of an infection induced in the laboratory. After being exposed on one occasion to the bites of infective tropical rat mites (*Liponyssus bacoti*), a prepatent interval of about 50 days occurs in infections which later reach moderate or high levels of intensity. The microfilariae then appear in the peripheral circulation. The rate of the increase in the count is proportional to the peak finally attained and in these high infections the microfilariae are usually present in the circulation for a year or more.

It is possible, therefore, to predict with some confidence the subsequent course of the infection, after having followed its course for the first 2 months, particularly in those cases in which the original rise is rapid. The course of several infections of varying intensities was shown.

If a drug which is effective against the adult worms, though not against the larvae, be given 2 months after the larvae have appeared in the peripheral circulation, then the upward rise of the infection will be arrested; for the larvae migrate from the adult worm to the peripheral circulation in a day or so, and as no more larvae will be produced, the number in the circulation will gradually fall until they finally disappear. The subsequent rise and the peak will thus be cut off and the course of the infection will be brought to a premature end.

On the other hand, a drug which is effective against the larvae will cause an immediate fall in the numbers of circulating larvae, which will then rise again as the effect of the drug wears off, and as new-born larvae are produced and complete their migration to the blood.

To confirm the effect of the drug on the adult worms the animals are treated in pairs, the infection in one animal being allowed to complete its evolution, and the other being examined after an interval of from 2 weeks to 1 or 2 months after treatment. In an animal which has had no treatment at this stage of infection, the adult worms are free and mobile, there is very rarely any surrounding reaction and the larvae in the pleural exudate are present in enormous numbers. In an animal to which a drug effective against the adult worms has been given, the adults are immobile and are more usually encapsulated in fibrin and endothelial cells and eosinophils, and the reaction may have undergone fibrous ... the larvae may be absent or very difficult to find and are immobile and immature.

Examples were shown from some preliminary experiments using MSb (Friedheim) in which a single dose of mg 250 per kilo body weight was given by intraperitoneal injection. From these results it would seem to be effective against the adult *Litomosoides carinii* and to have little action upon the larvae in the circulation.

MISCELLANEOUS

Dr R. H. P. Clark

X-ray films illustrating the value of pneumo-hepatography in the investigation of apparent liver deformities, with special reference to liver abscess. (Shown by Dr C. C. Chesterman.)

General Note.—

The intraperitoneal introduction of 300 to 500 ml. of oxygen has been

found adequate for good separation of liver and diaphragm This is completely absorbed in 2 to 3 days Radiography was performed in the erect position

Reference may be made to CLARK, R H P, & DUTTA, D K, *Ind med Gaz* 1945, **80,** 554 (abstracted in *Trop Dis Bull*, **43,** 559)

Four sets of films were exhibited

Exhibits

A and B—Films from two cases with normal upper contours of the liver In each a deformity appears before introduction of oxygen In the one case (Exhibit A) the liver "hump" is shown to be caused by marked digitalization of the diaphragm In the other (Exhibit B) tenting of the liver is of supra-diaphragmatic origin

C and D—Films from two cases of liver abscess Here the procedure is seen to assist in localizing the abscess, and in assessing the extent of the lesion

Dr C J Hackett and Mr W A Norman, F I M L T (Wellcome Museum of Medical Science, London)

The use of plastics in embedding and mounting specimens

Methods for embedding specimens in methyl methacrylate ("Perspex"), Wards "Bio-Plastic" and "Marco S B 26C" Resin, and for making perspex specimen containers were demonstrated

The techniques

(I) EMBEDDING IN METHYL METHACRYLATE ("PERSPEX")
(Imperial Chemical Industries)

Materials Methyl methacrylate monomer ("Kallodoc" I C I)
Benzoyl peroxide (catalyst)
Dibutyl phthalate (plasticizer)
Sodium hydroxide

(1) PREPARATION OF THE EMBEDDING SYRUP

(i) The monomer is washed, in a separating funnel, with an equal quantity of 5 per cent sodium hydroxide to remove the stabilizer, hydro-quinone, the discoloured NaOH solution is run off The washing is repeated

(ii) The alkali is removed by washing several times with distilled water, until washings are neutral to phenolphthalein

(iii) The monomer is dehydrated with flaked calcium chloride for 24 hours

(iv) It is then filtered and catalyst (0 1 per cent) is added

(v) Plasticizer (15 per cent) is added

(vi) The monomer is partially polymerized in a flask on a boiling water bath for 15 to 30 minutes The time depends upon the thickness of the syrup required This should be done with care as the reaction is exothermic and may cause complete polymerization To avoid this the monomer mixture is either continually stirred or carefully shaken at least four times Even with the latter method a violent reaction may occur (Note syrup thickens on cooling)

The final syrup can be stored for a few weeks at 0° to 4° C in the dark but polymerization slowly proceeds

(2) PREPARATION OF THE SPECIMEN

The specimen should be dehydrated after fixation, by taking through ascending grades of alcohols to absolute alcohol It is then placed in chloroform overnight, and finally into a thin syrup for penetration which may be assisted by lowering the pressure

The specimen is now ready for embedding. (Note: "perspex" embedding tends to clear biological material.)

(3) MAKING THE MOULD.

A mould can be quickly and easily made by joining suitably shaped pieces of glass with sernmentum to form a box. "Sernmentum" sets quickly and will hold the glass in place in a few minutes. The mould is either left overnight or may be completely dried and hardened in a 40° C. oven in a few hours. ("Sernmentum" is a fireproof tile cement made by BCM/SMNT, London, W.C.1.)

(4) EMBEDDING.

Embedding is best done in layers.

(i) A layer of syrup, about ¼ inch thick, is poured into the mould and polymerized at 40° C. for a few days, to form a supporting layer. (Note: time may be saved by pouring a *thinner first layer on to a sheet of ¼ inch thick* perspex on the bottom of the mould.) Polymerization at room temperature may be carried out by ultra-violet light.

(ii) Another layer of syrup is poured into the mould and the specimen set in position.

(iii) *Further layers may be needed to cover the specimen adequately if it is thick* and to allow for evaporation and incomplete polymerization.

A glass lid bound on to the mould with cellophane tape to form an air-tight cover should be used *during polymerization to prevent evaporation.*

(iv) When polymerization is complete the block can be shaken out of the mould or glass broken away. Polymerization takes 1 to 3 weeks.

(v) The block is cut and trimmed to size and shape and finally polished by hand or on a finishing machine.

(Benzoyl peroxide should be stored and handled with caution since should it become dry an explosion may be initiated by heat or movement. It is usually supplied containing 30 per cent. water and should be stored in one-ounce lots.)

(II) EMBEDDING IN WARD'S BIO-PLASTIC.
(Ward's Natural Science Establishment, Inc., Rochester 9, New York.)

Materials: Ward's Bio-Plastic ("Selectron").
Tertiary butyl hydroperoxide (Ward's catalyst).

For Opaque Specimens.

(1) The specimen is fixed and preserved in a formaldehyde fixative.

(2) Catalysed "bio-plastic" is prepared by adding 0·1 to 0·5 per cent. of Ward catalyst to the monomer. The amount of catalyst used *is proportional to the size or thickness of the layer.*

(3) The supporting layer is poured into the mould and allowed to gel at room temperature *for 1 to 3 hours.*

(4) While any bubbles are rising from the catalysed monomer the specimen is dried by blotting with absorbent material and then air-dried *just short of shrivelling and darkening.*

(5) A layer of catalysed monomer just sufficient to cover the specimen, is then poured.

(6) The specimen is carefully set in position so that no air bubbles are trapped. Gelling is allowed to occur at room temperature.

(7) The final layer *is poured and allowed to gel at room temperature.*

(8) Final polymerization is accomplished in an oven starting at 37° C. and gradually rising to, but not exceeding, 60° C. This takes 12 to 18 hours.

(9) The block is *removed from the mould, shaped and polished by hand or on* finishing machine.

To get a cleared specimen it should be dehydrated with alcohol and then placed in anhydrous ether. From this *it is transferred to uncatalysed monomer and the ether is* slowly removed in a desiccator by slowly reducing the pressure. (Note: too rapid evacuation can cause the ether to boil and damage the specimen.) The specimen is placed into catalysed *monomer and the above technique* followed.

(III) Embedding in "Marco S B 26C" Resin
(Scott Bader & Co , 109, Kingsway, London, W C 2)

Materials "Marco S B 26C" resin
"Monomer C"
H C H catalyst (1-hydroxycyclohexyl hydroperoxide-1)
"Accelerator E" (solution of cobalt naphthenate in "Monomer C")
Diethyl phthalate (plasticizer)

PREPARATION OF PLASTIC

(1) Mixture A "Marco S B 26C" resin 100 parts
"Monomer C" 20 parts
(2) Mixture B Catalyst 2 parts } warm to
"Monomer C" 20 parts } dissolve
(3) mixture of A and B is added
Plasticizer 10 parts
(4) This mixture is then filtered through glass wool It is stable for 1 to 2 days
(5) "Accelerator E" 1 part is added and plastic is ready for polymerization as in "Ward's bio-plastic"

Opaque Specimens and Clear Specimens

The remainder of the technique for opaque and clear specimens is the same as that for Ward's "bio-plastic"

The embedding of biological material in transparent plastics is still in the experimental stage and the ideal plastic has yet to be discovered Unless great care is taken to prevent undue temperature being reached during polymerization, especially when thick layers or blocks are being handled, internal stresses may result in damage to the specimens or in fissures in the plastic This difficulty may be avoided by lowering the temperature for polymerization or reducing the quantities of catalyst and accelerator used which will slow the rate of polymerization

(IV) The Preparation of "Perspex" Specimen Containers
Professor J B Duguid s technique (University of Durham)

Materials Clear sheet "perspex" 1/16, 1/8 and 1/4 inch thickness (I C I)
Ethylene dichloride

(Plastized sheet softens at 70° C and unplastized sheet at 110° C The latter may possibly be more suitable for transport to the tropics)

Summary of Technique.

The two sides and top are made of one bent strip, the front and back are sealed to this strip and then the base is sealed in position after the mounted specimen has been placed in the container Finally, the container is filled through a small hole in the base which is then plugged

(1) Cut a strip of 1/4-inch "perspex," of required width and length

(2) Heat and bend the two top angles Heating may be by a small gas flame moved in a guide beneath the strip or two electrically heated copper rods may be used

(3) Rest bent strip on small pieces of 20-amp fuse wire on a glass plate

(4) Run ethylene dichloride into space between glass and "perspex" and leave for 15 minutes, recharging when necessary

(5) Drain off surplus ethylene dichloride and place on a sheet of 1/16-inch sheet which is slightly larger than the front of the container

(6) Weight in position for several hours

(7) Trim off surplus with knife or saw

(8) Cement two small "perspex" stops on the inner surface of the sides towards back to retain mounted specimen in position

(9) Fix the other surface of container as above (3 to 7)

(10) Round edges and corners Polish by hand or machine

(11) Mount specimen on a piece of 1/8-inch sheet which is made to fit into the container

(12) Make and polish base plate from ⅛-inch sheet. Drill a small hole in this near but internal to the back of the container

(13) Place container on fuse wire as in (3) but over hole in the glass plate to allow pressures to equalise.

(14) Run ethylene dichloride under edges. Leave for 15 minutes, recharging when necessary

(15) Drain off and invert container *Carefully place mounted specimen in container* so that prepared edges of container are not touched.

(16) Place the base on to the prepared edges and apply weight for several hours.

(17) While the base plate is drying mounting fluid is run in through the hole until the specimen is just covered.

(18) When the container is dry completely fill with mounting fluid and remove air bubbles.

(19) Plug hole with tapered plug of perspex and seal off with "perspex cement. *For containers over 9 × 9 inches thicker sheet may be used.*

Dr F Hawking Lt.-Col. W Laurie Mr P Sewell, Ph.D Miss S Thurston B.Sc

Investigations on the antifilarial action of hetrazan on *Litomosoides, Wuchereria bancrofti* and *Onchocerca volvulus*

Photographs and slides were demonstrated showing the action of hetrazan on *Litomosoides carinii* of cotton rats and on *W bancrofti* and *O volvulus* in patients in East Africa. Hetrazan acts mainly on the microfilariae and has little action on the adult worms (*Litomosoides* and *Onchocerca*). The microfilariae disappear rapidly from the blood and are concentrated in the liver where they are destroyed by phagocytes. This work has been described in *Lancet* 1948, **2**, 730 and 1949 **2**, 146.

Dr M Lubran (introduced by Dr F Hawking).

The blood concentration and urinary excretion of hetrazan

A brief account of the method of hetrazan determination was given, together with some curves showing the effects of single and repeated *doses of the drug*

Dr C. A Hoare (Wellcome Laboratories of Tropical Medicine).

Races of *Trypanosoma evansi* produced by mutation.

Among the pathogenic mammalian trypanosomes there sometimes occur individuals lacking the characteristic kinetoplast (= kinetonucleus). These variants are especially common in *Trypanosoma evansi* their proportion in this species usually varying from 0·01 to 10 per cent. but sometimes fluctuating more widely

In addition to individual variation in the number of akinetoplastic forms found naturally in *T evansi*, totally akinetoplastic strains can be produced artificially by treating infected animals with certain organic dyestuffs. In such strains all the trypanosomes are deprived of the kinetoplast and never recover this organ.

It has also been demonstrated that akinetoplastic strains of *T evansi* may appear spontaneously both under laboratory and natural conditions. Thus, a normal North African equine strain (" *T marocanum* "), which had been maintained in laboratory rodents for 5 years, suddenly became completely akineto-

plastic (WENYON, 1928, HOARE and BENNETT, 1937, 1939) and retained this peculiarity for 17 years (1928–1945) Under natural conditions this phenomenon was observed in the Anglo-Egyptian Sudan, where the examination of over a hundred camels suffering from surra revealed in five animals infections with akinetoplastic trypanosomes exclusively, whereas the trypanosomes in all the other camels were normal as far as this organ was concerned (HOARE and BENNETT, 1937, 1939) One of the akinetoplastic cameline strains was isolated into laboratory rodents and has been kept under continuous observation for the last 13 years (January, 1937—November, 1949), in the course of which the aberrant condition has remained unchanged

It is thus seen that both induced and spontaneous akinetoplastic strains of *T evansi* have become permanently fixed, breeding true for an indefinite period In the case of akinetoplastic strains produced by the action of chemicals, the disappearance of the kinetoplast may be due to its direct destruction or to the loss of power to divide In the case of akinetoplastic trypanosomes arising spontaneously, the primary cause of the disappearance of this organ is unknown, but the mechanism by which the aberrant condition is perpetuated can be observed directly Thus, in normal strains of *T evansi* the kinetoplast of some trypanosomes fails to divide, with the result that after binary fission one daughter-trypanosome retains the parental kinetoplast and the other is devoid of this organ Both the latter and the trypanosomes in a totally akinetoplastic strain continue to propagate similar individuals All the available evidence, therefore, indicates that the kinetoplast once lost does not arise *de novo*

The loss of the kinetoplast in *T evansi*—both induced and spontaneous—represents a heritable variation having all the attributes of a mutation, for variants possessing the new character (absence of a kinetoplast) appear suddenly, breed true from the beginning, and give rise to a new strain or race, which becomes permanently fixed (HOARE, 1940)

The only example of the natural occurrence of a species of trypanosomes completely devoid of a kinetoplast is *T equinum*, the causative organism of Mal de Caderas in South America, which is indistinguishable from *T evansi*, except in this feature In the light of the discovery of spontaneous akinetoplastic strains in the last-named species, there can now be no reasonable doubt that *T equinum* also originated as an akinetoplastic mutation of *T evansi*, which had established itself in the New World and has continued to breed true as a mutant species for at least half a century since its discovery in 1901 (HOARE, 1949) Indeed, if one of the Sudanese camels harbouring an akinetoplastic strain of *T evansi* had been introduced into a country, where Surra was absent but susceptible hosts and suitable vectors were present, it might have given rise to a species identical with *T equinum*

The demonstration provided examples of the type of mutation described above

REFERENCES.

HOARE, C. A. (1940) *J Roy micr Soc* 60 29.
——— (1949). *Rec Sec M sc Hist nat.* (In the press.)
——— & BENNETT S. C. J (1937). *Parasitology* 29 43
——— & ——— (1939). *Ibid.*, 30 529
WENYON, C. M. (1928). *Trans R. Soc trop. Med. Hyg* 22 85.

Dr M H Hughes and Capt P J Daly R.A.M.C

Ocular Onchocerciasis

Sections of an eye, excised by Capt. P J DALY from a patient in the Gold Coast.

The patient bore numerous *Onchocerca* nodules, and showed typical skin lesions. The left eye, sections of which were shown, contained complicated cataract, and was blind and painful. Microfilariae of *O volvulus* were absent from the conjunctival biopsy but were present in the intraocular fluid after excision. The patient showed perception of light only in his remaining eye there were numerous microfilariae in the anterior chamber, there were patches of nummular keratitis, and also iris bombé choroido-retinitis, and optic atrophy

The sections showed microfilariae in the cornea, sclera, external layer of choroid and optic nerve which were not usually associated with inflammatory changes. There were patches of interstitial keratitis cellular infiltration of sclera, choroiditis, retinal degeneration and primary onchocercal optic neuritis. It was suggested that the well-stained microfilariae were living, and excited no tissue reaction, and that the inflammatory changes were associated with dead microfilariae which had not taken up the stain well. No microfilariae were found in retina, iris, or ciliary body and it is possible that the chronic scleritis interfered with the blood-supply and nutrition of the uvea sufficiently to produce the gross loss of function apparent in the patient remaining eye.

Dr H Lehmann

The nature of macrocytic anaemia in Central Africans

Blood slides, bone marrow smears, photographs of vitally stained blood and case histories were exhibited.

In Uganda the majority of anaemic patients show on admission to hospital a hypochromic normo- or microcytic blood picture. There is only poikilocytosis and anisocytosis. Unlike the iron deficiency anaemia usually seen in Europe and in India, in the African iron deficiency anaemia the emphasis lies more on hypochromia than on microcytosis consequently pessary cells predominate

On iron treatment the blood picture alters dramatically The anaemia becomes macrocytic. The cell count remains at first almost stationary the old pessary cells disappear and are replaced by large cells with normal mean corpuscular haemoglobin. The turnover of cell population suggested that destruction process goes on and iron merely alters the nature of the replacing cells. The new cells are larger than normal cells but carry the normal amount of haemoglobin per cell (M.C.H. is normal) they are consequently hypochromic as far as haemoglobin concentration is concerned, M.C.H.C is below normal. These cells are macrocytic-hypochromic this has until recently been taken to denote dual deficiency hypochromia → iron deficiency macrocytosis → liver factor deficiency It can be shown by vital staining that the macrocytic hypochromic cells are reticulocytes or early post reticulocytes. The macrocytic anaemia in Central Africa is thus due to the outpouring of reticulocytes and early post reticulocytes produced by an efficiently functioning bone marrow in an attempt to balance peripheral blood destruction (extra- and intravascular) Complete cure of the iron deficiency anaemia can only be achieved by removal of hookworms. Otherwise stationary phase with 9 to 12 grammes per cent. haemoglobin and mild reticulocytosis and macrocytosis, is encountered. Thus the true cause of iron

deficiency anaemia in Uganda is hookworms They are present in the human intestines in their thousands, and produce puncture wounds from which blood oozes forth Although most of this blood is rapidly digested and reabsorbed, organic iron compounds are notoriously non-available to absorption The patients become, therefore, rapidly iron deficient Iron treatment alone will cure only this aspect of hookworm anaemia, without hookworm purge a mild anaemia—slightly macrocytic—will remain and not respond to iron therapy

The macrocytic blood picture (which in iron deficiency anaemia only presents itself following iron treatment) is present initially in malarial anaemia or in anaemia of infection In these later conditions the blood is destroyed intravascularly and the iron is not lost to the body as it is in hookworm anaemia Thus the " precoctic " blood picture is present at the start Graphs and slides were shown which demonstrate macrocytosis and reticulocytosis in untreated malarial anaemia, and the concurrent fall of macrocytosis and reticulocytosis following antimalarial treatment

A slide was shown from a patient who received for 7 days both iron treatment and folic acid mg 5 t d s Macrocytosis and dual cell population were present in spite of folic acid treatment Nutritional macrocytic anaemia responds well to folic acid therapy, the macrocytosis seen in Africa is not due to nutritional deficiencies

Bone marrow smears were shown from patients who developed a macrocytic blood picture on iron treatment There were no megaloblasts present, this excluded deficiency of liver factor as cause of macrocytosis

Dr H Lehmann and Dr P W Hutton

Recovery of a fatty liver as demonstrated by serial biopsy

Monthly photographs and liver biopsy slides were shown from a 17-year-old Murundi patient admitted to hospital with anaemia, generalized oedema and a number of vitamin-deficiency symptoms

Pyrexia and a raised leucocyte count with numerous staff cells containing toxic granules as well as macrocytosis with a raised percentage of reticulocytes, suggested the diagnosis of precoctic anaemia due to infection Sulphadiazine treatment and a diet rich in calories and vitamins were of no avail, but after 2 months the removal of septic teeth halted the deterioration of the anaemia and within a further 5 months a haemoglobin of 14 4 g per cent was reached, as well as a substantial increase of the blood volume

On admission a fatty liver was noted and the recovery was followed in monthly biopsy specimens At first almost every parenchymal cell is distended with fat Vacuoles can be seen in the nuclei such as have been described in livers of animals exposed to anoxia (O A TROWELL (1946) *J Physiol*, **105**, 268)

Gradually the portal rim of the lobules becomes free of fat. Five months after admission half the liver tissue, that on the portal sides of the lobules, consists of fairly normal, non-fatty cells After 6 and 7 months the liver tissue is almost normal, an occasional fatty cell remains Portal tracts are in places normal, in other areas they show a slight increase in fibrous tissue There are present in places double nuclei and there is some inequality in cell size, but on the whole the cell picture is normal, there are no vacuoles in the nuclei Reticulin stains show that at this stage there is more reticulin present than seen in normal European livers, but there is no loss of lobular pattern, and considering the degree of initial fatty infiltration the amount of reticulin is much less than one might have expected to find

The importance of infections in causing diseases often associated with dietary deficiencies alone is emphasized

Mr P G Shute

Thin and thick films showing malaria parasites on the same slide and in the same microscope field

FIELD, in his monograph *The Microscopic Diagnosis of Human Malaria, I* states "For demonstration or teaching, the advantage of combined thick and thin films on one slide can be attained by mounting a stained thick film taken on a coverslip on top of a stained thin film on a slide. This method gives a convincing demonstration to students of the concentration factor in the thick film but it is too complicated for general use."

If preferred, the thin film can be made on a coverslip and the thick film on a slide. The thin films are stained with Baird and Tatlock s Leishman and the thick films by Field s method and after drying, by weak Leishman (one drop of stain to 5 c.c. of distilled water).

Dr G T Stewart

Experimental neuro-trypanosomiasis in the monkey

Monkeys were infected intraperitoneally or (better) intracisternally with 0·1 c.c. *Trypanosoma rhodesiense* suspension (washed and concentrated in Ringer s solution). The progress of the infection was followed by examining blood and C.S.F and by histological observations upon monkeys dying or sacrificed at various intervals after inoculation.

When monkeys were infected intraperitoneally a proportion succumbed in a few days with an overwhelming parasitaemia. The remainder survi ed for 3 weeks to 6 months. Those surviving for 2 months or more developed meningo-encephalitis. The early parasitaemic deaths were avoided by inoculating the trypanosomes directly into the cisterna magna, under nembutal anaesthesia.

Histological observations showed that infection of the central nervous system was established 2 to 3 weeks after inoculation. At this stage, there was an inflammatory reaction in the choroid plexus and meninges. After the 8th week, the C.S F showed increase in cells and protein, and contained trypanosomes. Lethargy somnolence and transient attacks of coma then occurred.

Monkeys dying early (5 to 8 days) showed heavy parasitaemia and splenomegaly but no changes in the nervous system. Those dying at about 3 weeks showed inflammatory changes in the choroid plexus and meninges, and generalized lymphadenopathy Those dying or sacrificed at 2 to 6 months showed meningoencephalitis with numerous trypanosomes in the choroid plexus, and isolated trypanosomes in the brain substance.

Experiments on guinea-pigs and rabbits, variously manipulated failed to induce with any consistency a definite infection of the nervous system. It is probable, therefore that the experimental chemotherapy of neuro-trypanosomiasis can best be studied in the monkey For this purpose a recently isolated strain of *T rhodesiense* was found most suitable as monkeys have a high tolerance for old laboratory strains of *T rhodesiense* or *T gambiense*

Dr. F Murgatroyd and Dr A. W Woodruff (Hospital for Tropical Diseases, University College Hospital, London)

The effect of "Banocide" (Hetrazan) on adult forms and microfilariae of *Loa loa*

The results of treatment with banocide (1-di-ethyl-carbamyl-4-piperazine) in cases of *Loa loa* infection have been described by MURGATROYD and WOODRUFF (1949) The demonstration illustrated two of these results (1) Death of the adult *L loa* worms (2) Disappearance of microfilaria loa from the blood.

Photographs were shown of adult *L loa* worms under the skin of a patient These appeared 24 hours after the commencement of treatment with "banocide" mg 6 per kg body weight daily One was excised after it had remained immobile in the skin for 26 hours and a second after a period of immobility for 7 days' duration The worms appeared to be dead when they were removed

A chart was shown demonstrating the effect of banocide on microfilariae of *L loa* and of *Acanthocheilonema perstans* in a patient with a double infection Before treatment the microfilaria counts in 20 c mm blood were *L loa* mean 294 (Range 280–309), *A perstans*, mean 112 (Range 101–119) Two days after the commencement of treatment with banocide (mg 6 per kg body weight daily) microfilaria loa disappeared completely from the blood During the course of treatment microfilaria perstans averaged 76 per 20 c mm blood (Range 48–109)

Blood films illustrating these changes were shown Mr A V H ALLEN had kindly prepared these before and after the commencement of treatment The former contained microfilaria loa and perstans, the latter microfilaria perstans only

REFERENCE MURGATROYD, F, & WOODRUFF, A W (1949) *Lancet*, 2, 147

Mr. J S Steward

Living specimens of *Hippobosca equina* L and its wingless relation *Melophagus ovinus* L

The Hippoboscidae are interesting as showing all gradations between complete winglessness and functional wings

Melophagus, the common ked of sheep, has no signs of wings (exhibited)

Hippobosca (horses, camels, etc) have good functional wings (exhibited) —as also *Ornithomyia* spp on birds

Lipoptena (on deer) are usually wingless—the wings having broken off

Other genera (*Crataerhina*, etc) on swifts and swallows have reduced non-functional wings

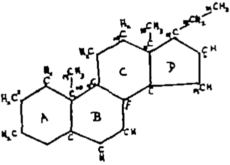
So far as can be seen *Hippobosca* in both horses and camels seldom use their wings, and it would appear as though, in the course of evolution, they will become wingless

Hippobosca equina L —These specimens were taken on 17th November in Cheshire from Dartmoor ponies which were bought in Devon on 10th October They have become less numerous in the last few weeks They are common in the New Forest and are recorded from Scotland—probably occurring where horses run wild The females deposit mature larvae which immediately pupate

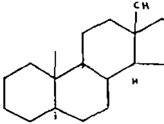
lymphopenia and decrease in the number of lymphocytes in lymphoid tissue together with concomitant *increase in serum protein*, chiefly *globulin*. Injection of either hormone into rabbits, previously immunised with sheep erythrocytes, produced an *increase in titer within six hours and return to original level, after 24 hrs.* Extracts *of lymphocytes* contained both antibodies and protein with the mobility of *gamma-globulin* the presence of gamma-globulin in lymphocyte extracts, was demonstrated immunologically by Kass. Injection of either hormone into rabbits immunised with sheep erythrocytes but with serum titers, allowed to decline, produced a sharp anamnestic rise in antibody titer within 5 to 10 hrs. no such response could be elicited by antigen. WHITE and collaborators interpret these phenomena as indicating release of antibody from lymphocytes during this interval, under hormonal influence.

The structural nuclei of the two hormones, as appearing in the parent, saturated hydrocarbons of the adrenal steroids, are (1) Allopregnane and (2) Androstane, the stereochemical formulation and system of numeration of which are given below

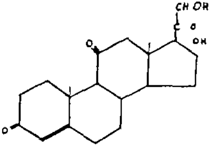
(1) *Allopregnane*



(2) Androstane



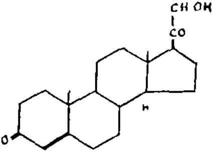
17-Hydroxy-11-dehydrocorticosterone (Kendall's Compound E)



and the Adrenocorticotrophic Hormone (ACTH).

The Adrenal-Cortical steroids are compounds of the following types

Desoxycorticosterone



Immunization in human trypanosomiasis could be attempted on the same line by administering (1) *sheep erythrocytes* on rabbits and (2) 2nd stage experimental monkey erythrocytes injected into man, followed by injection of either hormone (adrenocortical and pituitary adrenocorticotrophic) as derived from the herewith described (keto) steroids.

REFERENCE

KABAT E. A. (1946). Immunochemistry *Ann. Rev Biochem* 15

TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE
Vol 43 No 4 January, 1950

ORDINARY MEETING

of the Society held at
Manson House, 26, Portland Place,
on
Thursday, 7th December, 1949, at 7 30 p m

THE VICE-PRESIDENT,
Professor BRIAN G MAEGRAITH, M B , B S , D Phil
in the Chair

PAPER

TROPICAL PHLEBITIS AND OTHER POSSIBLY RELATED VASCULAR DISORDERS IN TROPICAL AFRICA

BY

MICHAEL GELFAND, M D , M R C P ,*
Physician, Salisbury Native Hospital, Southern Rhodesia

I am honoured and, at the same time, grateful for the opportunity of addressing you I might add that I have looked forward to the day when I, like so many before me, could make my contribution, however insignificant, to our Society

To those of us in the tropics, London is still our Mecca—the heart from which spring the arteries, from whence comes our blood supply Some of you may have expressed fears as to the future of this centre, but speaking as one from the periphery, I am more than ever sure that we in the Colonies need you just as an orchestra needs its conductor You are better placed for facilities for the finer lines of research Last, but not least, the inspiration and spirit of this Society still pervade the whole of the tropical world, both within and without the Empire

The spirit of PATRICK MANSON is still here I cannot help feeling that these lines of Kipling, written for Cecil John Rhodes, the founder of my country, could well be used of MANSON'S influence on tropical medicine

> " The immense and brooding spirit
> Still shall quicken and control ,
> Living he was the land
> And dead, his soul shall be her soul "

* I wish to express my gratitude to my house physician, Dr M P MAVROS, for his assistance in the wards, to Prof F FORMAN, Cape Town University, for his advice and criticisms, and to Dr R M MORRIS, O B E , M D , Secretary for Health, Southern Rhodesia, for his kind permission to publish this address

It is with some trepidation that I present the subject of tropical phlebitis, as I am not clear in my own mind as to the status of this disorder in tropical medicine. Nor am I certain of its relationship to the many other vascular disorders now being described from all parts of the world. It does not appear it is true, to be connected with these latter disturbances, such as periarteritis nodosa, temporal arteritis, thrombo-angiitis obliterans, dermatomyositis, diffuse lupus erythematosus, scleroderma and phlebitis migrans. To establish a disease as an entity is not easy for even after it has been described as such confirmation must be obtained from others.

Very little is available on tropical phlebitis in the literature. Indeed, there are scarcely half a dozen articles relating to this disorder and less than that number of workers have written on the subject. I hope you will forgive me if my name seems to appear somewhat more frequently than is usual. It is not because I wish to push my point of view but that, as there have been so few writers on this disorder I have had to put forward my ideas in order to corroborate or disagree with the observations of other workers. The disease, which may affect many different veins, is claimed to have a distinctive pathology with inclusion bodies in particular cells. In time, workers in or outside tropical Africa will probably be able to confirm that the condition first described by FISHER in Northern Rhodesia as a disease entity is indeed such, and does not—on the other hand—belong to some other recognized group of disorders. Consequently it is hoped that those taking part in the discussion this evening will be able to criticize freely the evidence that has so far been presented: namely that in the tropics, but more particularly in the sub-tropics of Africa, there exists a peculiar affection of the veins and possibly as I shall mention too, of the arteries. It is only by such frank criticism that those of us working far from the larger centres, with little opportunity of discussion, may be re-directed into correct channels when we have assumed a wrong course. Perhaps, too, certain suggestions may be made as to approach or research which may clarify the issue. Thus it may be revealed that what has been defined as a particular group of tropical disorders, hitherto regarded as distinct entities, may after all possess a common basic aetiology

HISTORY

As you are all well aware, non-suppurative thrombophlebitis may be primary or secondary. Secondary thrombophlebitis may be the result of trauma local infection, general debilitating diseases or fevers, or may follow operation, cardiac ailments or childbirth (phlebothrombosis).

In primary thrombophlebitis or thrombophlebitis migrans, which is relatively uncommon, widely separated regions of the body are affected. Its cause is unknown, there being no relation to pre-existing disease or trauma. Pulmonary thrombosis often occurs but complete recovery is usual.

There have been references to a phlebitis of tropical origin in the literature

CASTELLANI (1930) referred to the existence of a condition he called periphlebitis tropica, but a careful study of his note in no way differentiates this from ordinary thrombophlebitis migrans which LOW and COOK described a year later as occurring in a lascar fireman and a Malayan seaman

LOW and COOK state "The two cases illustrate the simplest variety of thrombophlebitis migrans, in which only the veins of the extremities are affected, without implication of the viscera Otherwise as is characteristic of thrombophlebitis migrans no aetiological factor could be elicited"

An apparently new form of primary phlebitis—tropical primary phlebitis—was first reported in 1941 by FISHER, who published an excellent account of the condition in the *South African Medical Journal* His cases, mostly African but occasionally European, were encountered in the copper mining area of Northern Rhodesia He referred to the disorder as "acute thrombophlebitis of unknown aetiology," describing its histological features accurately and suggesting that it might be a disease entity In 1943, having become acquainted with FISHER's paper, I published a clinical account of the disease, mentioning some of the many veins which may be involved Three years later I again recorded findings similar to those of FISHER, and included two European cases in this series I described a case of femoral thrombosis with pulmonary embolism, although he agreed that embolic phenomena are very rarely seen in the disease

A most useful publication was that by MANSON-BAHR and CHARTERS in 1946 who, for the first time, published from East Africa in the *Lancet* a comprehensive clinical account of the disease The paper was entitled "Epidemic thrombophlebitis" and in it is recorded the astounding number of cases—627—admitted to the No 1 and No 3 (East Africa) General Hospitals between January, 1944, and December, 1945 They describe three clinical varieties of the disease, the emphasis being on phlebitis of the neck veins They are the first to report on the relapsing nature of the complaint and to record its association with arteritis and gangrene They make the interesting suggestion that the disorder might possibly be a virus infection transmitted by needle-puncture, as a high percentage of the cases had recently undergone anti-syphilitic treatment

Perhaps the most valuable of FISHER's contributions was that in which he was associated with his wife, Dr MONICA FISHER, and with Prof A C LENDRUM This was published in the *Journal of Pathology and Bacteriology* in 1946 Here, for the first time, was provided an accurate and detailed description of the pathology of the vessel wall and the demonstration, by a special staining process, of inclusion bodies in the polyblast cells The postulation that so-called splenic abscess might be related to tropical phlebitis is put forward and the suggestion made that the term "idiopathic thrombo-phlebitis" be replaced by "primary tropical phlebitis" This paper should be consulted in the original as it also clarifies the disease from the clinical aspect

In the same year I published papers in the *Transactions* of the Royal Society of Tropical Medicine and Hygiene and the *Lancet* also postulating a relationship

between primary splenic abscess and primary tropical thrombophlebitis. In this connection, one might mention that I recorded a case in 1948 of multiple splenic infarction associated with splenic thrombosis. This patient also showed a mesenteric thrombosis with small bowel complications. Further information bearing on this subject is offered by my publication in 1949 of a case of total splenic necrosis, in which both the vein and the artery were thrombosed.

Perhaps a step farther in the history of this interesting disorder was my report on six cases of symmetrical gangrene of the feet and toes, seen in African males (1947). In each patient oedema preceded the gangrene. I was unable to offer an explanation for the cases. In a letter to the *British Medical Journal* in 1948, however CHARTERS and MANSON BAHR suggested that these cases might be related to "that condition known as tropical thrombophlebitis." Further they refer to their own two cases of arteritis with gangrene of the foot. My cases were strictly symmetrical both in time of onset and extent, whereas those of CHARTERS and MANSON BAHR were unilateral. Later however I published two cases in which only one of the upper extremities was affected, the digits alone being involved. The gangrene was preceded by oedema in all the cases. Although not entirely convinced, I thus lent support to the view of CHARTERS and MANSON-BAHR that the condition described might, in fact, be that of thrombophlebitis with secondary arterial disease or spasm. Evidence of possible arterial involvement is submitted in a paper published in the *South African Medical Journal* (1949), in which I mention the fact that in cases of femoral thrombosis, the oedema of the limbs was relieved to a certain degree by a lumbar-sympathetic block. In other papers (1949), cases of tropical myositis are described and the suggestion is made by me that perhaps one variety of this condition may be related to tropical phlebitis.

PATHOLOGY

The three papers dealing with the pathology of the disorder are by FISHER (1941), FISHER, FISHER and LENDRUM (1946) and MANSON BAHR and CHARTERS (1946). Of these the most important is that by the FISHERS and LENDRUM which portrays in detail the histological picture of the disease in th veins. Its features vary it would appear from case to case, apparently depending on the stage reached by the process at the time the biopsy was performed. They stress the gross destruction and upheaval in all coats of the veins but more especially in the media, the layers of which are disrupted and widely separated or fragmented by an actively proliferative oedematous vascular tissue. FISHER and LENDRUM believe that the new interrupting or granulation tissue has its origin in the dividing zone between the intima and media, the latter then being invaded. This tissue consists mainly of fibroblasts or endothelial cells, giant cells remin iscent of the large giant cells of Hodgkin's disease, or of the foreign body type capillaries and fibrous tissue. In addition a varying number of polymorpho-nuclear leucocytes, whose forms are often well preserved, is commonly found.

Other chronic inflammatory cells, particularly lymphocytes and plasma cells, may form part of the general picture, as pointed out by the late Dr F W SIMSON, of the South African Institute of Medical Research, when reporting on one of FISHER'S earlier cases He particularly mentioned that the vasa vasorum, although dilated and engorged with red cells, showed no obvious cuffing by inflammatory cells

FISHER and his colleagues comment on the short stretch of the wall which is damaged, the thrombus at this site being firm, white, of a fleshy appearance and strongly adherent to the inner lining of the vein Distal to the thrombus and for a considerable distance from it, secondary clot formation due to stasis of the blood supply, supervenes These authors, as well as MANSON-BAHR and CHARTERS, also refer to the eventual organization with recanalization of the thrombus, or the complete and permanent obliteration of the lumen by dense fibrous tissue

Whereas no bacteria or other organisms, such as rickettsiae, were demonstrable, by various staining methods, in tissue removed from the patients, LENDRUM and FISHER (1946) claim to have detected, in some of their tissue preparations, cytoplasmic inclusion bodies within the large endotheliod, or what they refer to as polyblast cells These bodies were stained strongly red by the phloxintartrazine method They were circular in outline and of fairly dense hyaline appearance The bodies were scanty in number, not more than four or five being present in a transverse section of the whole vein at the level of maximum development of the lesion As far as I am aware no further work on this particular aspect has been carried out to corroborate their findings

It is not my wish to comment to any extent on the histological findings which were similar to those found by me in sections taken from material removed either by biopsy or at autopsy However, a few points should be stressed as they may have some bearing on the pathogenesis One of the most interesting features to strike me was that in addition to the marked degree of phlebitis in which all three coats are seriously affected by the chronic granulating process there is—as will be seen from the sections I have prepared—a clear and obvious infiltration of the surrounding connective tissues of the wall of the vein for a variable distance beyond it The inflammation in the tissue in the neighbourhood of the vessel may be as intense as it is in the wall itself The tender swelling felt clinically is in fact usually thicker than that which one would ordinarily associate with a thrombosed vessel alone

The other significant observation is that the clot in the vessel often appears recent in spite of the extensive fibrous and new tissue formation which has occurred in the vascular and perivascular tissues It would suggest that for some time a chronic inflammatory lesion had been smouldering in the vessel wall and then, for some unknown reason, the thrombus suddenly supervened (DE NAVASQUEZ, personal communication) Does this peculiar infection commence in the immediate vicinity of a vein and spread inwards from without the vessel wall ?

An artery too may be involved should it be lying nearby Owing to the greater thickness of its coats, however it seems to be able to resist the infection to a greater extent than can the relatively thinner and weaker venous wall. Is this after all a disease primarily of the connective tissues, affecting particularly those in the close vicinity of the vascular system, or may not such inflammatory tumours appear occasionally in sites unrelated to the vascular system? This is merely a point of view since this is not as a rule seen clinically the patient not admitting to any previous tenderness of long duration in the site where the inflammatory process is taking place. He complains only of a sudden pain and tenderness, with the swelling of the limb supervening within a day or two of the commencement of the illness. I cannot explain the discrepancy between the pathological picture and the clinical history (Figs. 1 to 6.)

Clinical Features

Much can be written on the clinical picture mainly because of the many different veins involved and because the inflammation, in a relapse, may attack another vein.

The essential feature in a typical or average case is the more or less sudden onset of a febrile disturbance in an otherwise healthy young African, who complains almost at the same time of pain of a severe nature over the affected vein. If for instance, the femoral vein is involved, the pain will be situated over the groin and down the front and side of the thigh. Sooner or later generally within a few days, peripheral oedema, varying enormously from case to case, appears. The amount of swelling probably depends on the extent of the venous occlusion and possibly on the degree of the arterial spasm. The fever is still maintained and there may be pronounced constitutional disturbances. After several or more days the oedema tends to subside. (Figs. 7 and 8.)

When discussing the pathology I referred to the tender inflammatory mass in or over the vein. In the early stages it may be exquisitely tender and varies much in size. It is fixed to the subcutaneous tissues and in my experience does not in itself suppurate. It gradually diminishes in size over several or many days, revealing in this event the outline of the thrombosed vessel as a cord-like firm structure the length of which varies according to the extent of the thrombosis.

The site of oedema depends on the particular vessel thrombosed. A vein commonly involved is the subclavian or axillary when oedema of the upper extremity and of the shoulder is pronounced. Thrombosis of the jugular vessels may cause swelling of the affected side of the neck, face and eyelids. In 1949 I recorded a case of this type in which both external jugular veins were occluded. On account of the distribution of the oedema, it was mistaken on admission for Bright's disease. The main leg veins may be clotted, producing swelling in the calf and foot. A not uncommon thrombosis is that of the superior vena cava,

1 —Biopsy taken on a saphenous vein in the
n It illustrates the occlusion of the vessel
thrombus and the extensive inflammatory
ges in its wall, particularly the adventitia
extending beyond into the surrounding
tissues

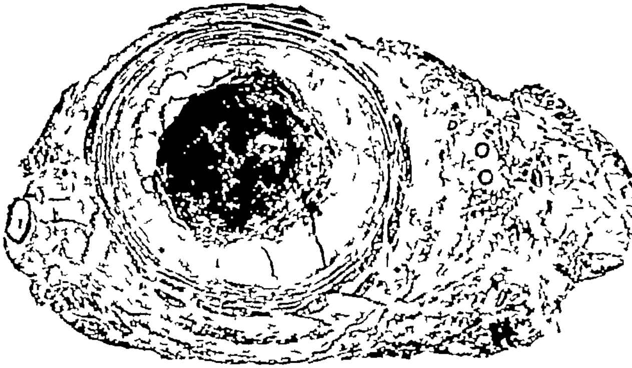


Fig 3—Another biopsy performed on a small saphenous vein in t
calf It demonstrates well the pronounced perivascular inflammati

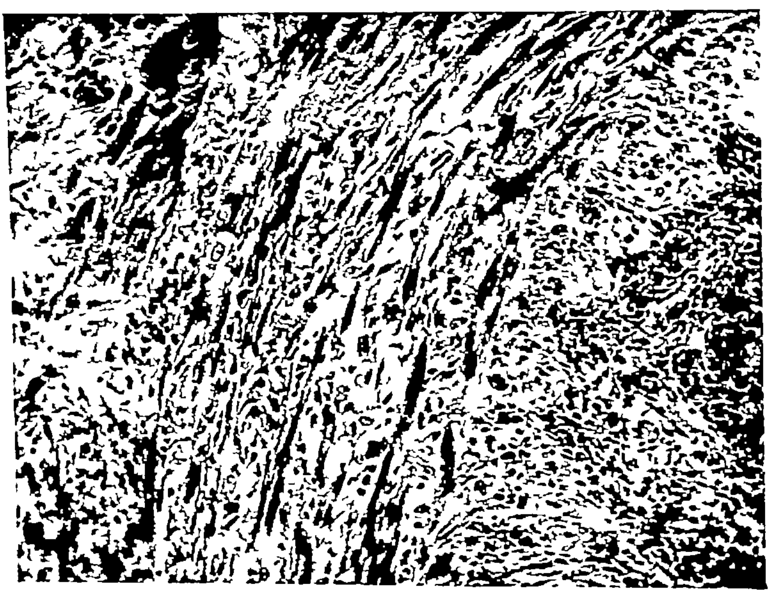


Fig. 2—In this view of the saphenous vein a pronounced cellular reaction has occurred in the vessel wall, the medial coat being fragmented

To face page 374

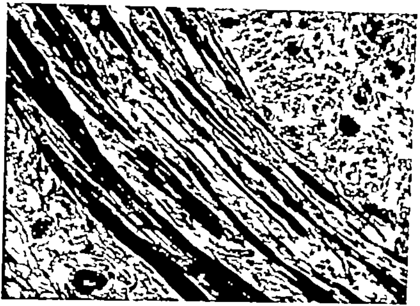


Fig. 4.—In this case the cellular reaction in the medial layer is comparatively mild as compared with that present in the intimal and thrombus region. Organization of the clot is taking plac

Fig. 5.—Phlebitis in small saphenous vein, illustrating the thrombus together with inflammatory changes in the vessel wall extending well beyond its confines

Fig. 6.—The splenic vein and artery are occluded by large thrombi. There is an extensive inflammatory process surrounding and between each vessel. The entire splenic tissue within the capsule had undergone hyperplastic necrosis.

Note particularly the damage to the arterial and

Fig 7—Showing left femoral thrombosis

Fig 8—Acute phlebitis of the right popliteal vein Note the definitive swelling of the leg especially of the ankle and foot.

when the oedema is seen to affect both upper extremities, the face and neck The prognosis with regard to life I have found to be remarkably favourable

An interesting syndrome seen in practice is one in which thrombosis of the inferior vena cava occurs This results in extensive oedema of both lower extremities, the pubic regions and the front and back of the lower abdominal wall Such oedema, as is the case when the superior vena cava is blocked, may persist for a long period The swelling may gradually disappear or continue indefinitely It is possible that some of the African cases, seen from time to time, with enlarged or tortuous veins coursing upwards across the abdomen and trunk, result from the establishment of a collateral circulation due to the thrombosed vessel, and not to be confused with that produced by hepatic cirrhosis and portal obstruction (Fig 9)

It does not follow, however, that there is always oedema when a vessel is

affected. For instance, in spite of the fact that the thrombosed vessels in case could be felt, no surrounding oedema was found distal to or in the vicinity of the vessels. This may be accounted for by the good collateral drainage of the area or simply by the fact that no actual thrombosis had occurred in the lumen. Another possible explanation might be the slight degree of arterial spasm present.

As the oedema in the non-idiopathic variety of thrombophlebitis tends to disappear with a lumbar sympathetic block, I carried out this procedure in cases of tropical phlebitis of the femoral vein. Novocaine was injected into the lumbar sympathetic ganglia. The oedema subsided rapidly as a rule within 48 hours. This would seem to suggest that the oedema is due not only to venous obstruction but also to some degree of arterial or arteriolar spasm through sympathetic action.

An interesting outbreak of tropical thrombophlebitis was seen in the Salisbury Native Hospital from April to June, 1949 during which time 22 cases were admitted. In the months before and after this period only sporadic cases

FIG. 9.—Prominent varicosities on the abdominal wall. No hepatic or splenic disease found on clinical examination (although his loss does not exclude these). This may have been the result of thrombosis of the inferior vena cava with the subsequent establishment of collateral circulation. The earlier history was suggesti

were encountered. No explanation can be offered for this. Of the 22 cases, all were males except one. The average age of the patients was 30 years, the oldest being 40 and the youngest about 20. Out of the 15 patients questioned, 12 admitted that they had recently received injections—these being for syphilis in every case except one. All recovered except the sole female who died of a portal thrombophlebitis. The functional recovery was excellent in 19 but the remaining two left hospital with a slight swelling of the leg

In nine of the patients, more than one vein was involved—generally two

As a rule the multiple thrombophlebitis started first in one vessel, affecting another a little while later The sites attacked in the total series were as follows

(1) The femoral vein in six cases, one of which was multiple

(2) The cervical in eleven patients, in six of whom other veins were affected as well

(3) The calf and foot were swollen in seven cases

(4) The portal vein was occluded in one patient In addition a cervical vein was involved

(5) Two cases were of the nodular variety

The intensity of the fever ranged from 99° to 103 4° F , the average being 101 5° F Such pyrexia was seen in all the cases Its duration varied from 1 to 20 days, the average being 5½

White cell differential counts were performed on ten cases Five of them were normal In three a leucopenia with a relative lymphocytosis was shown, and in two a moderate polymorphonuclear leucytosis Blood cultures in four cases proved sterile

In a series of 105 cases, MANSON-BAHR and CHARTERS found the following veins involved

Vein	Number of cases	Vein	Number of cases
One internal saphenous	15	One superficial arm vein	16
Both saphenous	4	Superficial arm veins (right and left)	4
One femoral	22	Portal	2
Both femoral	21		
One popliteal	21		105

Of these patients six had phlebitis of both arms and legs

GELFAND (1946) reported on 15 cases, the majority of which showed involvement of the femoral vein The subclavian was also attacked in a few of the cases In the same series was a case of mesenteric involvement, one with the splenic vein and another with the cavernous sinus thrombosis

In a series of 32 cases, FISHER (1941) found the veins involved as follows

Vein	Number of cases	Vein	Number of cases
Cavernous sinus	1	Portal	2
Internal jugular	6	Femoral	10
External jugular	2	Popliteal	1
Subclavian	2	Short saphenous	2
Axillary	5		
Basilic	1		32

The average duration of illness in this series was 33 days, the range being from 5 to 91 days

affected. For instance, in spite of the fact that the thrombosed vessels in case could be felt, no surrounding oedema was found distal to, or in the vicinity of the vessels. This may be accounted for by the good collateral drainage of the area or simply by the fact that no actual thrombosis had occurred in the lumen. Another possible explanation might be the slight degree of arterial spasm present.

As the oedema in the non-idiopathic variety of thrombophlebitis tends to disappear with a lumbar sympathetic block, I carried out this procedure in cases of tropical phlebitis of the femoral vein. Novocaine was injected into the lumbar sympathetic ganglia. The oedema subsided rapidly as a rule within 48 hours. This would seem to suggest that the oedema is due not only to venous obstruction, but also to some degree of arterial or arteriolar spasm through sympathetic action.

An interesting outbreak of tropical thrombophlebitis was seen in the Salisbury Native Hospital from April to June, 1949 during which time 22 cases were admitted. In the months before and after this period only sporadic cases

FIG. 9.—Prominent varicosities in the abdominal wall. No hepatic or splenic disease found on clinical examination (although this alone does not exclude these). This may have been the result of thrombosis of the inferior vena cava with the subsequent establishment of collateral circulation. The earlier history was suggestive.

were encountered. No explanation can be offered for this. Of the 22 cases, all were males except one. The average age of the patients was 30 years, the oldest being 40 and the youngest about 20. Out of the 15 patients questioned, 12 admitted that they had recently received injections—these being for syphilis in every case except one. All recovered except the sole female who died of a portal thrombophlebitis. The functional recovery was excellent in 19 but the remaining two left hospital with a slight swelling of the leg.

In nine of the patients, more than one vein was involved—generally two.

As a rule the multiple thrombophlebitis started first in one vessel, affecting another a little while later. The sites attacked in the total series were as follows

(1) The femoral vein in six cases, one of which was multiple

(2) The cervical in eleven patients, in six of whom other veins were affected as well

(3) The calf and foot were swollen in seven cases

(4) The portal vein was occluded in one patient. In addition a cervical vein was involved

(5) Two cases were of the nodular variety

The intensity of the fever ranged from 99° to 103 4° F, the average being 101 5° F. Such pyrexia was seen in all the cases. Its duration varied from 1 to 20 days, the average being 5½

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Basilic	1		
			32

The average duration of illness in this series was 33 days, the range being from 5 to 91 days

In a series of 68 cases reported by FISHER *et al.* (1947) the following veins were attacked superior sagittal sinus, cavernous sinus, internal jugular external jugular popliteal, subclavian, axillary basilic, portal, femoral, short saphenous. Fourteen of the patients had multiple lesions.

The prognosis is usually much more serious when visceral veins are inflamed and thrombosed, largely on account of interference with a vital function. Death may not infrequently result from such an attack. The chief thrombosed visceral veins to have been recorded are

> Superior mesenteric (FISHER and GELFAND) splenic (FISHER and GELFAND) portal (FISHER and MANSON BAHR and CHARTERS) sagittal sinus (FISHER) cavernous sinus (FISHER and GELFAND).

Perhaps the most common of these to be affected is the superior mesenteric vein or one of its radicles. If the parent trunk is involved, the patient develops an acute abdominal attack with severe epigastric and umbilical pains, shock vomiting and often with the passage of reddish brown blood per rectum. This disorder may frequently be mistaken for acute volvulus (a common condition in the African), acute intussusception (also frequent in him) and other abdominal emergencies.

When a small radicle of the superior mesenteric vein is attacked, the prognosis is better In such an event only a small portion of the small intestine becomes deeply congested. The clinical picture is much milder and may be confused with that of an acute dysentery In fact the clinician may wonder whether or not to open the abdomen. In one such case, a tender mass could be felt in the umbilical region, but thrombophlebitic changes were present in other parts of the body The severity of the attack and the shock are much less than when the main vessel is thrombosed.

The picture produced by a portal, cavernous sinus or sagittal sinus vein thrombosis is sufficiently well known not to warrant its repetition. It is on the splenic vein, however that I wish to dwell a few moments. Cases of splenic venous thrombosis are recorded by GELFAND (1946) and FISHER *et al.* (1947). The two effects of thrombosis in the vein are

(1) Multiple infarction of the spleen.

(2) Total necrosis of the splenic pulp (a more advanced change).

(1) *Multiple Infarction of the Spleen.*

The diagnosis is not easy but is suggested by the severe pain in the splenic region and the exclusion of other causes of pain over this organ. This condition may be suspected, if in addition to the pain it was preceded, accompanied or followed by venous thrombosis in other parts of the body In many cases, however the diagnosis is made only at operation or autopsy

(2) *Total Splenic Infarction and Splenic Abscess*

When total splenic infarction occurs the picture is one of severe pain over the spleen, usually with a palpable splenic tumour FISHER (1947) and GELFAND (1947) postulated that so-called primary splenic abscess might be a later manifestation of total splenic infarction with liquefactive necrosis

Three other papers published this year deal with primary splenic abscess in Africans The first is by JELLIFFE who reviews the subject and records two cases from Nigeria The second is contributed by BEET, whose case, a Northern Rhodesia Native, had sickalaemia coincidentally, and the third by SINGER reporting on the occurrence of the disease in a Southern Rhodesia Native

It is, perhaps, advisable at this point, for the purpose of clarity, to mention briefly the subject of primary splenic abscesses This condition was first described by WALLACE (1922), from Broken Hill, Northern Rhodesia, when he reported a large number of Native cases, seen over a comparatively short space of time, with huge splenic abscesses He was unable to find a cause, such as an amoebic infestation, either clinically or at autopsy Since he was sufficiently observant to note that many of the patients had also developed thrombosis of the leg veins, he suggested that there might be a thrombosis of the splenic vein. It appears clear to me that WALLACE's cases were in reality what FISHER was to recognize some time later as tropical phlebitis

In 1949 I published an interesting case of phlebitis and secondary arteritis of the splenic vessels with total liquefaction of the splenic pulp A large quantity of blood was found in the splenic capsule at autopsy I suggested that primary splenic abscess might be a later development of total splenic infarction

Gangrene in the Extremities

Gangrene in an extremity, particularly of the fingers or toes, has been linked with tropical phlebitis Cases which might possibly be related to the phlebitis as well as to an arteritis were published by MANSON-BAHR and CHARTERS (1946) and GELFAND (1947, 1948 and 1949) The former writers described two cases of tropical phlebitis in patients who later developed gangrene in an extremity, necessitating amputation GELFAND reported on two types of cases In the first, the gangrene of the toes and feet in Africans was symmetrical (Fig 10) Oedema preceded the gangrene which soon followed and appeared simultaneously in toes or feet of both extremities, each being affected equally in both extent and degree In the second type the gangrene was limited to one side and as a rule involved the fingers In the case shown in illustration (Fig 11) the fingers and toes are absent on each of the four limbs The patient, in this case, was an elderly Native aged about 60 who, 5 years previously, was tilling his land when he suddenly experienced severe pain, first in the forearms and hands These became oedematous and later he lost the digits About 2 weeks later a similar attack developed in the feet The onset of the illness in this case is typical of

Fig. 10.—Symmetrical gangrene involving both feet. The patient was young native male, previously in excellent health, who suddenly developed pain and swelling of each foot followed by gangrene

Fig. 11.—In this case described in the text the patient developed sudden swelling in both hands with loss of few toes. This was followed by similar attack some time later involving the feet, with loss of few of the digits

this type of gangrene—namely, sudden pain with oedema, followed soon af by the gangrene. It should be mentioned, *à propos* of this case, that other caus of gangrene such as diabetes, heart disorders and embolic disease, were sati factorily excluded. There was nothing to suggest leprosy. Ergot poisonin had been carefully eliminated, and in any event this type of gangrene has no been encountered, as far as I am aware, in the Native.

I should at this stage refer to confirmatory reports on the subject of symmetrical gangrene from other parts of tropical Africa. One is by SALTER (1947) who described an interesting case in an Ethiopian, and others are by SNELL from Uganda and BLOSS from the Sudan, both in 1948. Except for minor differences, such as for instance in SNELL's case in which all four extremities were involved, these cases were similar to mine. All the patients were male, the ages varying from about 25 to 35 years. In none could any obvious causation be determined. All enjoyed good health prior to the onset of the gangrene and there were no signs suggestive of malnutrition.

A recent description (HUGHES, 1949) of an African female from the Gold Coast, alleged to have had thromboangiitis obliterans, is of interest. I cannot help wondering in view of the extreme rarity of this disease in the female, whether the case might not in fact have been more closely related to the condition which I am describing.

One cannot definitely claim that these peculiar cases of gangrene in the African belong to tropical phlebitis or angiitis. Pathological proof of the nature of the disorder is lacking, but its interesting onset, with oedema of the limb, followed by a peripheral gangrene usually confined to the digits and only occasionally extending to the foot or hand, is perhaps suggestive that such cases may fall into the category of tropical phlebitis. On the other hand, symmetrical gangrene may belong to an entirely different entity.

The question as to whether or not tropical phlebitis and tropical myositis are related is still less certain. As is well known, there are several causes ascribed to tropical myositis. Some favour *Staphylococcus aureus*, others the filarial parasite. Others again would prefer an initial haemorrhage into the muscle from an ascorbic acid deficiency, followed by infection of the haematoma. We know, however, that whilst it is not unusual to isolate an organism from the pus or fluid, it is not uncommon for the culture to be sterile. *A propos* of filarial disease, it is not seen in some of the areas in which myositis tropica is described. I do not wish to imply that these various causes may not account for some or perhaps most of these cases, but merely to point out the possibility that a few of them may be caused by tropical phlebitis, as a result of the cutting off of the arterial supply, as well as the venous. In the same way as in the case with splenic arterial and venous thrombosis, a corresponding disease of the vein and/or artery serving a muscle may, perhaps, at a later stage, result in muscle

oedema. Sometimes thickened, tender cord-like thrombosed vein can be felt. After the fever has subsided, there may be relapse of fever later accompanied by thrombophlebitis in another limb, or by stiff neck or even without localizing signs. The thrombosed vein, if localized sometimes persists for many months as thickened, hard, fibrosed cord.

(ii) *Subacute Thrombophlebitis*

Some patients are admitted with no other manifestations than oedema of one or both legs with irregular pyrexia. Pain is often absent and the thrombosed vein is not palpable

(iii) *Unusual Varieties.*

(1) *Chronic*.—They describe two cases which developed recurrent bouts of localized venous thrombosis every 3 weeks for 5 months. During each attack the pyrexia lasted 2 to 3 days and small nodule could be palpated along the course of superficial vein. In each case biopsy of the nodule revealed an organizing thrombus in the vein.

(2) *Portal Vein Involvement*

(3) *Association with Arteritis.*—They record two cases admitted to hospital with phlebitis which developed arterial thrombosis with resultant gangrene. In each case the leg had to be amputated from above the knee after which recovery took place

CLASSIFICATION (C).

Taking into account the variable clinical picture I would suggest the following classification

(1) *The Oedematous Type* (Phlebitis major), due to phlebitis of major peripheral vessel. A particular part of the body depending on the vessel thrombosed, is involved, *e.g.* the leg arm or face

(2) *The Acute Abdominal Variety* (Phlebitis and Thrombosis of visceral vessel, such as the mesenteric, portal and splenic veins and including splenic abscess or infarction of the spleen).

(3) *The Cerebral Type* with thrombophlebitis of cerebral vein and cavernous sinus, closely simulating meningitis, encephalitis or resulting in focal paralyses such as a hemiplegia.

(4) *Phlebitis Minor* affecting small superficial veins such as of the neck, leg and arm.

(5) *The Relapsing Type.*—Here the patient recovers, but later relapses, another vein being attacked.

(6) *The Spreading Type*.—In this variety the disease spreads to other veins at the time of the illness or shortly after

(7) *The Nodular Type* in which multiple small pea-like swellings due to phlebitis of superficial minor vein occur

(8) *Tropical Myositis* (one variety only) (?).

(9) *Phlebitis and Arteritis*

( ) Unilateral gangrene of an extremity (?)

(*b*) Symmetrical gangrene of the extremities (?).

(10) *Acute gangrene of skin* (?) *e.g.* Fournier disease and perhaps tropical ulcers

DIAGNOSIS.

The main features of this condition are

(1) It is predominantly a disease of the African.

(2) Males are mostly attacked, often those who are relatively young and in good general health.

(3) Pyrexia of varying intensity is present.

(4) When a peripheral vein is occluded, distal oedema is the rule
(5) The disease is seen throughout the year, but small outbreaks may occur from time to time

In cases in which a superficial vein is involved, the diagnosis can be established by a biopsy Where the vessel is more deep-seated and such procedure, therefore, considered unwise, as with the femoral artery or popliteal veins, palpation of the tender swelling following the course of the vein suggests that the vessel is affected

The diagnosis is made mainly on the clinical picture as already described, after the exclusion of other possible causes for thrombosis of a vein, such as thrombophlebitis after typhoid, typhus and relapsing fevers, pneumonia, malaria and that following upon an operation or childbirth (phlebothrombosis)

The disease may resemble a number of different conditions, as will be appreciated on studying the many diverse features of the disease and the number of veins likely to be affected It may simulate most of the acute febrile disturbances, such as malaria, pneumonia or meningitis, or the long-continued pyrexial illnesses, particularly typhoid fever When there is pain and tenderness in a limb it may closely resemble acute ostiomyelitis, poliomyelitis or scurvy Epidemic myalgia, especially of the cervical muscles, may resemble the disease There is a number of acute abdominal disturbances already mentioned that may produce a clinical picture similar to that of tropical phlebitis when one of the main abdominal veins is attacked

COMPLICATIONS AND SEQUELAE

Most cases recover, but at times certain complications and sequelae follow Embolism is rare Neither FISHER nor MANSON-BAHR and CHARTERS have encountered it, and I have seen it only once The patient was a young African male who developed a femoral thrombophlebitis, and several days later coughed up blood There was a patch of consolidation at the base of the right lung (Confirmed radiologically)

Persistent oedema of varying degree in the limbs is a complication which is recognized by both FISHER and MANSON-BAHR and CHARTERS However, whilst I agree with them as regards the femoral vein, I have not as yet encountered it with thrombosis of the subclavian or axillary veins

A sequela mentioned by FISHER *et al* is enlargement of the collateral veins Several interesting effects, as pointed out earlier, may follow this

Suppuration is not generally found, but I have suggested that occasionally necrosis of the muscle with abscess formation may follow the onset of the disease

AETIOLOGY

One very important observation on the aetiology of the disease has been made I have already referred to the publication by FISHER, FISHER and LEN-RUM, in which they announce the existence of inclusion bodies in the fibroblast

or polyblast cells. This work has not yet been confirmed by others. Such a line of investigation should be undertaken by workers encountering the disease in tropical Africa.

An interesting observation, mentioned earlier in this paper is by MANSON BAHR and CHARTERS (1946) who noticed that the majority of their patients with thrombophlebitis had recently undergone a course of arsenic injections for syphilis. Most of them had previously received a venupuncture. Of 143 questioned, 120 had been injected mostly with N.A.B. intravenously. The remaining 23 gave no history of an injection. The interval between the last injection and the onset of symptoms varied from 3 days to 7 months.

On the whole, MANSON BAHR and CHARTERS favour a virus aetiology. They consider the points in favour to be the relative lymphocytosis in the blood negative bacteriological findings and its possible aetiological and epidemiological relationship to the outbreak of infective hepatitis in their Command. The admissions to No. 2 General Hospital for infective hepatitis, at the time of this outbreak, were recorded and a similarity noted between the two curves of incidence. Moreover most of the cases of infective hepatitis were so-called post arsphenamine jaundice whilst undergoing anti-syphilitic treatment. They state "The theory that the present syndrome may be caused by a virus transmitted chiefly by needle puncture, but also by other means, such as droplet, urine or faeces, is attractive." They exclude all other causes of thrombophlebitis, such as marantic thrombosis. They mention that thrombosis of limb veins occurs in malnourished persons, but most of their patients were healthy African soldiers of Category A.

I have recently investigated the question of previous injections in my cases. Out of 15 with definite thrombophlebitis, 12 gave a previous history of injections, mostly of neoarsphenamine for syphilis.

Thus, at first sight, there appears to be strong evidence that as propounded by MANSON-BAHR and CHARTERS, this form of thrombophlebitis is of virus origin, most probably introduced by venupuncture.

However one wonders why venupuncture should produce a virus infection only in tropical Africa. Millions of injections are being given continually throughout the world. Why is it that this type of thrombophlebitis is not produced as a result of injections in other lands? I therefore decided to investigate this question further. As the African is so riddled with disease might it not be possible that at any time a large percentage of patients in an African hospital may have recently received injections for other disorders?

Accordingly 147 patients in the Salisbury Native Hospital in April, 1949 were questioned as to whether or not they had received treatment by injection during the period from September 1948 to April, 1949. It was found that 128 had received injections, mainly for syphilis, during those 6 or 7 months. Nineteen had not.

Syphilis, which is so prevalent in the African, might be considered as an

aetiological factor, especially as many of the patients seen either give a previous history of the disease or show a positive Wassermann reaction in the blood. On the other hand, it is not likely to produce such a pathological affect on the veins as the brunt of the attack falls rather on the arteries. Further, in many the Wassermann reaction is negative.

A point of interest in this disorder is that it is confined almost entirely to males, usually relatively young (between the ages of 20 and 45), and healthy. Females are rarely affected. I have seldom seen a typical case in a female, and not one in a child. FISHER *et al.* also report that it is not encountered in children or old people. Europeans may be affected, but only occasionally (FISHER, 1941; GELFAND, 1943).

With regard to the epidemiology, little can be said. There is suggestive evidence that it may occur in epidemic-like form or assume epidemic proportions. For instance, in MANSON-BAHR and CHARTERS's cases, the outbreak assumed its peak in the third quarter of 1947 (July to September, 204; October to December, 175). In all, there were 627 cases in 2 years.

FISHER (1941) noticed no seasonal incidence, but a tendency for several cases to occur within a few weeks of each other.

I find the disease tends to appear in small outbreaks, although sporadic cases are seen more or less throughout the year. In the present study all the 22 cases occurred within April and May of this year (1949).

In concluding this address, I hope I have presented sufficient evidence of the existence in South Central Africa of a common phlebitis which involves many veins and affects essentially young African males previously enjoying excellent health. It is almost always accompanied by a pyrexia of varying severity.

As far as I am aware, this entity appears to be confined to tropical Africa. As to whether the name "tropical phlebitis" is justified, I am undecided. The condition may be a primary affection of the veins, or one in which the granulomatous process commences in the connective tissues in the vicinity of a vessel, which later becomes involved. Until more is known about this disorder, however, it seems reasonable to retain the name of "tropical phlebitis" in the same way as the terms "tropical myositis" or "tropical ulcer" are employed. I have included in this address other conditions, some clearly vascular, others indecisively so, which are possibly related to tropical phlebitis. I mention these, however, largely to stimulate interest from a fresh angle, in a group of tropical disorders which perhaps deserve greater attention than has hitherto been accorded them.

REFERENCES

BEET, E. A. (1949). *E. Afr. med. J.*, 26, 180.
BLOSS, J. F. E. (1948). *Brit. med. J.*, 2, 52.
CASTELLANI, A. (1930). *Trans. R. Soc. trop. Med. Hyg.* 24, 379.
CHARTERS, A. D. & MANSON-BAHR, P. E. C. (1948). *Brit. Med. J.*, 1, 520.
FISHER, A. C. (1941). *S. Afr. med. J.*, 15, 131.
——, FISHER, M. M. & LINDRUM, A. C. (1947). *J. Path. Bact.*, 59, 105.

GELFAND M. (1943 & 1947). *The Sick African*. Cape Town: Stewart.
——— (1946). *Clin. Proc* **5** 331
——— (1947) *Lancet* **2**, 904
——— (1947). *Trans. R. Soc. trop. Med. Hyg* 40 789
——— (1947). *Brit med. J* **1** 847
——— (1949). *S Af med J* **23** 129
——— (1950). *Trans. R. Soc trop Med Hyg* I the press.
——— (1950). *Ibid.* I the press.
HUGHES, M. L (1949). *Ibid.*, 42 621
JELLIFFE, D B. (1949). *J trop. Med. Hyg* 52 134
LOW G C. & COOK, A. B. (1931). *Lancet*, 1 584.
MANSON-BAHR, P E. C. & CHARTERS, A. D (1946) *Lancet*, 2 333
SALTER, J G (1947). *Brit med. J* **1**, 108.
SPICER, R. (1949). *S. Afr med. J* **23**, 715.
SNELL, D G (1948). *E. Afr med. J* **25** 407
WALLACE, A. F (1923) *Med. J S Afr* 17 155

DISCUSSION

Dr S De Navasquez Considerable interest in the pathology of peripheral vascular disease has been stimulated in recent years by the wider recognition of cases of what appear to be spontaneous inflammatory lesions of arteries and veins of widespread distribution, best typified by periarteritis nodosa. While the histological changes in the latter condition are characteristic, they cannot be considered entirely specific, as similar changes may be seen in the necrotizing arteritis of malignant hypertension.

When Dr GELFAND showed me his sections, I was struck with the similarity of many of the changes in the veins to what one sees in periarteritis, although certain features of the latter condition are absent. The most conspicuous changes to be seen in the affected veins in Dr GELFAND's cases are firstly an inflammatory reaction of the adventitia and of the surrounding fibrous and adipose tissues, consisting of polymorph leucocytes, macrophages and fibroblasts, which appeared to be the oldest part of the lesion indicated by the presence of fibrous granulation tissue which extended far out into the perivascular connective tissue. Secondly swelling of the media, which appeared thicker than normal and showed an increase in the intercellular ground substance in between the muscle cells, most of which were still intact though foci of inflammatory cells were occasionally present. Finally the changes in the intima consisted of diffuse intimal thickening which, in some specimens, was as great as the total thickness of the media. Where the intimal endothelium had been shed, there was recent thrombus which was organizing This thrombus formation appeared to be localized to certain areas of the circumference of the

vein, and in no specimen was there complete occlusion of the vein The lumen invariably contained fresh red cells which were clearly still in circulation The arteries appeared normal, though the periarterial connective tissue was involved in the chronic inflammatory reaction Such is the microscopy of the lesion The interpretation is difficult in the absence of specimens stained to show elastic and reticulin tissues, but it is safe to assume that the oldest and therefore the original lesion is one of the perivascular and adventitial connective tissues, namely, a periphlebitis, and that the disease progresses from without inwards to involve the media and intima The thrombosis is limited to mural plaques which seldom extend to occlude the lumen and, as the organization of the thrombus is absent or incomplete, it is probably of more recent origin and not progressive, as is the thromboangiitis obliterans of Buerger

The differences between the lesions of tropical periphlebitis and the well recognized inflammatory diseases of peripheral blood vessels, such as periarteritis nodosa in which the veins may occasionally be involved and Buerger's disease, are clear cut There is no evidence of the fibrinoid necrosis affecting the intima and media which is so characteristic of periarteritis or of the progressive thrombosis indicative of the severe intimal damage in Buerger's disease

It is noteworthy that this tropical disease is for the most part confined to the veins and this may provide a clue to the pathogenesis by suggesting some extravascular agent capable of penetrating the less protected thinner wall of the vein, compared with the more robust and thicker walled artery, such as one sees in experimental procedures which involve the use of caustics on the skin when the veins bear the brunt of the injury and the arteries escape

One is naturally hesitant to accept the presence of "inclusion bodies" as evidence of a virus infection as too frequently such bodies have been shown to be artifacts There is one aspect of the "inclusion bodies" described by LENDRUM and FISHER which merits consideration and that is, their localization to cells which they call "polyblasts," and which they term reacting, suggesting thereby that they result from the lesion If such is the case, is it not surprising that the infective agent should be confined to reacting cells which are, presumably, late comers to the field of injury? It would have been more convincing if such bodies had been demonstrated in cells normally present in the wall of the vein

In view of the sharp localization of the disease to healthy young males, the possibility of an "occupational" aetiology arises The wide discrepancy in sex incidence also opens a further line of enquiry, which may be less fruitful The rarity of Buerger's thromboangiitis obliterans in women still awaits an explanation

Dr. D R C Willcox · I am glad to have the opportunity to take part in this discussion, for during the time I spent in Southern Rhodesia I saw many of these cases, although we did not then recognize that they formed such

a varied but distinct disease entity as Dr GELFAND has revealed to us tonight I did not meet the condition in epidemic form such as has been described since but there is no doubt that tropical phlebitis is met with sporadically in moderate numbers in all parts of the colony

Regarding the aetiology I was interested to hear Dr DE NAVASQUEZ stress the similarity to polyarteritis nodosa. I was myself struck by the similarity of some of the cases showing a superficial spreading gangrene to the condition described by SHELDON as purpura necrotica. Here the lesions start as areas of purpura, but necrosis and dry gangrene follow with eventual sloughing The lesions are peripheral and often symmetrical the prognosis, as in tropical phlebitis, is usually good. SHELDON suggested that the lesions were allied to the Schwartzman phenomenon and most cases had some focus of chronic sepsis. Others have suggested that these cases are a manifestation of polyarteritis nodosa.

The background of chronic sepsis is unlikely to apply to tropical phlebitis which occurs in young and otherwise fit male natives. Nevertheless, there appear to be many similarities between this group of conditions which Dr GELFAND has shown may well be related to tropical phlebitis, and the varied manifestations of the polyarteritis nodosa group. It may also be that there are aetiological factors in common. Bearing in mind the association of many cases of polyarteritis with the previous use of sulphonamides, and remembering also the popularity of these drugs with the native, it might perhaps be worth while to investigate a similar association of sulphonamides with tropical phlebitis.

Dr J Harper I would like to give some of my impressions on the epidemiology of perhaps 100 cases in askaris of tropical thrombophlebitis, seen for a short period in the early stages of their illness, during the 1944–45 outbreak in East Africa Command. (Some of these cases were later included by MANSON-BAHR and CHARTERS in their report in the *Lancet* (1946, 2 333).)

The syndrome occurred, in my experience, exclusively in those who had had intravenous arsenical injections for syphilis at the Special Treatment Centre to which my cases of venereal disease in the infectious stage were evacuated. Denial of such a history by the patient was always disproved either by later admission on persuasion, or by consultation of unit and hospital records. As one of the speakers has suggested the possible role of sulphonamides in the aetiology of this syndrome, it is of interest that the syndrome did not occur in cases of gonorrhoea, chancroid or lymphogranuloma inguinale treated with sulphonamides at the same time and at the same Special Treatment Centre.

It did not occur in a few cases of syphilis treated with intravenous arsenical in my depot, nor in cases treated there with intravenous arsenicals for relapsing fever (tick borne) It did not occur in cases subjected to venupuncture for

diagnosis, nor among the many hundreds of protective inoculations given weekly It had not occurred in many hundreds of cases given similar antisyphilitic treatment in Madagascar during the period 1942–44

It would be interesting if the waning of this epidemic in 1945 could be correlated in time with the changeover from intravenous arsenicals to intramuscular penicillin as the standard treatment for syphilis, as this changeover occurred also in 1945

The condition had three (possibly four) main features which occurred in all possible combinations and permutations These features were recurrent pyrexia, thrombophlebitis with oedema occurring in arm, leg and, possibly, neck (if visceral cases occurred they were misdiagnosed), and jaundice which was must less characteristic

We adopted the well-established principle in tropical medicine when dealing with a disease of relatively local and restricted incidence, and of obscure aetiology, and gave it a local habitation and a name, so that these cases became "S T C fever, neck, jaundice, leg or arm" Commanding officers were less exact and called it the "curse"!

Diagnosis after the first few cases was easy—and was often made by unit officers or African nursing orderlies on history and general appearance C S meningitis was easily separated as its main incidence was in recruits from Uganda, the stiff neck was bilateral, and lumbar puncture and reaction to sulphonamides were decisive Relapsing fever cases had a very different temperature chart Jaundice was more difficult to assess, because we had to consider, with having used a certain transit camp, and presented a close connection grafted on to the basic non-parasitic liver pathology of the African, virus diseases such as yellow fever, infectious hepatitis from Middle East personnel, syringe-transmitted jaundice, and direct hepato-toxicity of the arsenicals used, some of which were Italian "loot" of doubtful manufacture

Mr E G Tuckwell Dr Gelfand has described many cases of tropical phlebitis and I am struck by the similarity of them to a more acute phase of thromboangiitis obliterans as we see it. Many cases of thromboangiitis obliterans start with attacks of thrombophlebitis of the superficial or deep veins, the inflammatory process appears to begin in the perivascular tissues, possibly lymphatics, and spread through the walls of the vessels to produce a typical cellular thrombus This reaction is seen in segments of the vessels, normal structures being between the areas of inflammation

Has Dr Gelfand observed any relationship between tropical phlebitis and tobacco smoking? One has seen several cases of axillary vein thrombosis in healthy young men, who appear to have initiated the process by some quite mild exertion but of a type they do not usually use Such cases seem to recover completely, as did Dr Gelfand's cases I believe they are caused by tearing a tributary to the axillary vein to initiate the thrombotic process

Dr C C Chesterman stated that his only positive contribution to the discussion was to express his appreciation of the painstaking way in which Dr GELFAND had presented something comparatively new out of Africa. The restricted and localized epidemiology of the syndrome was confirmed by his own negative experience in the Congo. True, the sulphonamides were not in general use then, but countless injections of arsphenamine were being given without any evidence of thrombophlebitis.

He asked whether the Weil Felix reaction and the Frei s skin test had been used and whether there was any marked eosinophilia which might suggest a nematode role.

Tropical myositis had one point of similarity the pain and induration of the early lesion before any marked tension due to pus had developed.

Dr C J Hackett Dr GELFAND has hinted at the possible relationship between tropical phlebitis and tropical ulcer Has he found any radiological evidence in bone lesions which might have arisen from vascular interference in the former resembling those reported in association with the latter by BROCKLEBANK (1943. *British Journal of Radiology* 16 221) from West Africa and SHEPHERD (1946. *British Journal of Surgery* 83 352) from India?

Dr F Murgatroyd May I ask whether sickling was found in any of the patients?

Dr Gelfand (in reply) It would be difficult or impossible to reply in the short time at my disposal to the many interesting points raised in the discussion. We appear to have traversed the whole field of vascular pathology from thrombo-angitis obliterans to periarteritis nodosa. I, personally do not favour these two disorders (even though there may be points of similarity in the pathology), in view of the different course taken by tropical phlebitis which, as a rule, tend to attack a single large vein, a recurrence not usually being seen. In the relapsing type, there is generally only one relapse occurring within a short interval of the onset of the illness, and not a repetition for months or years. Further the prognosis is extremely favourable unless a vein serving a vital function is occluded. Temporal arteritis may possibly be more akin to the disorder I am describing this evening

The suggestion that ainhum may have a vascular pathology is a fascinating one, although it is difficult to accept that the dense fibrous constricting band is originally the result of obliterative vascular disease.

I have not observed any association between sickalaemia and tropical phlebitis. Nevertheless, as I have already mentioned in my paper BERT of Northern Rhodesia, recently recorded a case of splenic abscess showing the sickling phenomenon in the peripheral blood. I think the two are coincidental.

We have considered the possibility of a typhus-like infection of the veins, but the conclusion reached, largely from the Weil-Felix agglutination reaction, was that there was no relationship between a rickettsial infection and the pathology in the vessel wall

I am attached to the suggestion that tropical phlebitis may be allergic I cannot comment further on this point beyond mentioning that a marked eosinophilic response exceeding that usually met with in our African—is not the usual finding

A point worth reporting here is that tropical phlebitis in Africa would appear (with exceptions of course) to follow a similar geographical distribution as onyalai, although I must at once admit these two diseases are quite distinct and apart Why the two Rhodesias should see most of the cases I cannot say

With regard to bone changes in tropical ulcer, I have not observed a periostitis or osteitis, although in the more extensive cases infection of the underlying bone may quite possibly ensue

My main object in drawing attention to this fascinating disorder or syndrome was to interest other medical officers who might be here on leave and who on their return to the tropics might keep a wary eye on any such possible vascular phenomenon If I have achieved this I shall be glad

In conclusion, I should like to thank those who contributed to the discussion, and also my audience for bearing so patiently with me during the address

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After the discussion, the CHAIRMAN asked Dr L W HACKETT if he could give the meeting any information regarding the present malaria position in the United States Dr HACKETT said that an extraordinary decrease in reported cases of malaria had been occurring in the United States over the last 4 or 5 years, amounting on the average to about 45 per cent. a year, as shown by the following figures from 13 "malarious" States

1945	Approximately	50,000	cases	reported
1946	„	37,000	„	„
1947	„	14,000	„	„
1948	„	8,000	„	„

It is expected that the 1949 figures will show a similar trend

MACNAMARA wrote on a number of subjects, which are enumerated on page 147 of the Roll of the I.M.S., by D. G. CRAWFORD. By far the most important are his books on Asiatic Cholera, 1870 and on the History of Asiatic Cholera 1876 with a later supplement to 1892, which occupied him for many years. It was compiled from the Presidency Medical Board's records from the earliest date to show the spread of cholera over India and much of the world year by year and enabled the writer to work out maps of the world wide cholera pandemics of the nineteenth century for his book on Cholera and its Treatment of 1911 with full acknowledgements of his indebtedness to MACNAMARA's laborious work. This is acknowledged in a pathetic letter from MACNAMARA thus "It is the first and only reference I know of to my labours on the subject. I wrote when Bryden [J. M. statistical Officer in Simla], Cuningham [J. M. Surgeon-General to the Government of India 1880–85] and Fayrer [Sir Joseph, Medical Adviser to the Secretary of State for India] were all powerful with the Government and scoffed at my insisting on water carried cholera and advocated air propaganda pandemic waves and so on. I did not get a word of encouragement or help, but you have vindicated my conclusions for which I am thankful and extremely grateful."

In the course of his prolonged studies of cholera MACNAMARA recorded an instance in which 19 persons were known to have drunk some water which recently had been contaminated by a fresh rice water cholera stool, five of whom developed cholera. This convinced MACNAMARA that the disease was caused by an intestinal bacillus and led him to carry out animal experiments on the causation of cholera. In the course of these MACNAMARA contracted the disease and had to go home—a disappointed man—preparatory to retirement in 1876 from the Indian Medical Service, whose leading administrative officers had all "scoffed" at his prolonged investigations on by far the most important of the epidemic diseases of India. The distinguished surgical positions he held in London after his retirement from India once more illustrate the proverb that a prophet is not without honour save in the country of his life's work.

After his retirement from India in 1876 MACNAMARA not only retained his hope of being able to verify his hypothesis that cholera was caused by bacteria gaining access to the digestive system through contaminated water but also took active steps further to qualify himself to complete his long studies of the disease by proving his theory. For this purpose he went to Berlin to study the then comparatively new science of bacteriology under its leading exponent, Professor ROBERT KOCH and awaited a suitable opportunity. A careful continued watch informed him that cholera had spread from India to Aden and Mecca during the second half of 1882, and early in 1883 the disease appeared in Egypt as he expected. Accordingly on 9th February 1883, he submitted an application to the Secretary of State for India (see p. 397) asking for facilities to go to Egypt to carry out the bacteriological investigation for

which he had qualified himself and asked for the assistance for a few months of a recently recruited highly qualified Indian Medical Service officer The rest of the sad story can best be told in a letter to the writer dated 5th August, 1915, and the appended copy of his correspondence with the India Office, which he forwarded to me with the request that I would print it in a further edition of my book on cholera or in some other publication The following are the relevant passages in his letter

" After labouring at cholera for 20 consecutive years in Bengal I contracted the disease when experimenting on animals I came home and soon after went to study bacteriology under Koch in Berlin, with the knowledge thus acquired I was full of confidence in offering in March 1873 [this should read February 1883] to go to Cairo to work out the bacteriology of the disease Please read the enclosed correspondence, which do not return, and you will understand why an Englishman and one of the Indian Medical Service had not the privilege of discovering the cholera bacillus The Secretary of State's refusal on Fayrer's recommendation to accept my offer crushed any hopes I had of completing my life's work The enclosed correspondence shows what we are to expect for much anxious work "

(COPY)

'From N C Macnamara

To the Under Secretary of State for India

13, Grosvenor Street, W

Febry 9th, 1883

Sir,

In 1866 I published a work on Asiatic Cholera in Calcutta and have since written a volume on the subject which book has gone thro' two editions From my researches I believe Cholera to depend upon organic infecting matter, in fact, that like Scarlet fever and Smallpox, it is a disease which is communicable through the means of a specific poison contained in the fomes passed by patients suffering from Cholera I have lately taken steps to enable me to carry on further investigations into this important subject, having been urged to take this step because Professor Koch of Berlin has recently demonstrated the specific Bacilli of tubercle, and so much has been done within the past few years in this direction, that I think the time has arrived that fresh investigation as to the existence of a specific Cholera Bacillus should now be undertaken Professor Koch has proved the existence of tubercle Bacilli To carry on a work of this kind I should require some assistance, and from personal knowledge of Mr A Leahy I do not think a better man could be found to help me in such an investigation Mr Leahy lately gained more marks I believe than any man has yet done entering on the course of instruction at Netley—he passed his first examination with honours on the 5th inst, and is now therefore available, if the Secretary of State for India would grant him six months leave I cannot help feeling that he might institute a scientific investigation which I hope would lead to valuable results Mr Leahy is a good linguist, and might at once proceed to Berlin and work for a month with Professor Koch, in the meantime I shall probably be in a position to commence the examination of the specific organic matter passed by Cholera patients Under any circumstances I do not think these investigations would detain Mr Leahy more than

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six months, and beyond his pay and allowances for that time, the Government would be put to little expense. I believe if the Secretary of State refers this subject to the Medical Authorities at Netley they would bear me out in recommending Mr Leahy as a fit person for the duties I propose, and they would also I think testify as to the great importance of this work."

At the time of writing the above letter I knew that Asiatic Cholera had appeared in Egypt and had been in communication with my friend Mr Alonzo Money then living at Cairo, on the subject. My idea was to have gone to Egypt to study the bacteriology of Cholera evacuations. I had become acquainted with Professor Koch in Berlin and worked with him in his book on Asiatic Cholera he writes praising the results of my conclusions regarding this disease. I sent a copy of Sir J Fayrer's memo and my letters of February and March 1883 to Professor Koch. In the summer of 1883 Koch, with efficient assistants, at the German Government's expense, was sent to Cairo to undertake work I had proposed to perform earlier in the year but my offer was declined by the Secretary of State for India in consequence of his having received the following memorandum on the subject from Sir Joseph Fayrer who then occupied the position of Medical Adviser to the Indian Government."

(COPY)

"MEMO dated India Office Whitehall March 3rd, 1883 written by Sir J Fayrer Consulting Medical Officer to the Government of India, in reply to my letter to the Under Secretary of State for India, on the subject of Asiatic Cholera, dated Grosvenor Street, February 9th, 1883

The Etiology of Cholera is subject of great importance which is not by any means settled. Mr C. Macnamara advocates the theory that an organic contagion is the cause of Cholera and it is the investigation of the presumed form that he proposes to pursue. He is an able and energetic enquirer and worker has had large experience of the disease in Calcutta and his opinions therefore are entitled t respectful consideration.

For my own part experience leads me to assign the origin and predisposition (?) of Cholera to wider and more general causes. Our knowledge is as yet too limited to justify dogmatic assertions with regard to any theory of causation. I am well aware that contaminated water plays an important part in determining Cholera, and many other diseases, but I am not convinced that it does so because it contains specific organic poison. The question has been most carefully patiently and scientifically investigated (in all its aspects) for some years by Drs. Lewis and Cunningham with totally negative results, still, it is possible despit their special training and care that they may have failed t find that which in reality exists.

The investigation as proposed by M Macnamara would have t be carried on in London with imported Cholera discharges—Supposing the minute organisms anticipated be discovered how far they will be ascertained as the cause of disease which killed 313 people in Madras in 1874 and 97 050 in 1875 it is hard to say in the actual stage of theory and opinion which is prepared to ascribe even such disease as phthisis with ll its hereditary and personal peculiarities to imular cause say an organic germ, bacillus, I with others would regard these bacilli rather as epiphenomena than as being cause But as the out come of the microscopic researches now so actively pursued must be for good by tending

to settle an important question—I should be sorry to discourage investigation and would rather recommend that all important enquiry should be encouraged by the State especially when it regards so important a subject as that of Cholera etiology At the same time I would deprecate any action that interfered with the work of sanitary administration which must be based on ascertained facts alone

I should have thought that an investigation of this nature could have been carried out in India, the seat of the disease, and where all the means of working and staining bacilli might be done If this could be done in this country, I believe that Mr M is well qualified for the work and that no more able and zealous advocate of the subject of enquiry could be found, though I confess I do not share his expectation that it will reveal the cause of the disease

Should the Secretary of State in Council deem it expedient to renew the experiments of 1863 and desire to detail a highly qualified young medical officer for the purpose of being trained in Germany as recommended by Mr M to investigate Cholera organisms, I believe Surg Leahy possesses all the qualifications to fit him for such a duty

(Signed) J Fayrer, 3rd March, 1883

P S I added that I thought the advantage if any of conducting the enquiry in this country would be that it is done under Mr Macnamara's supervision'"

(COPY) "India Office, S W
9th April, 1883

M 1245

Sir,

I am directed by the Secretary of State for India in Council to acknowledge the receipt of your letter of the 12th February (*sic*) and to acquaint you in reply that on a careful consideration of your recommendation that Surgeon A Leahy should be allowed to remain in this country for a period of 6 months before proceeding to India in order to investigate Cholera in conjunction with yourself, the Earl of Kimberley has decided not to adopt your suggestion

I am,
Sir,
Your obedient servant,
ALLEN JOHNSON,
Major General, Military Secretary

Surg Maj N C Macnamara,
13, Grosvenor Street, W"

CONCLUSION

It only remains to point out that in many severe cases of cholera the causative organism is present in so nearly a pure culture, that it is readily isolated from the growth of streak cultures made direct from a cholera stool, to make it clear that a pupil of KOCH himself, such as MACNAMARA was, could hardly have failed to discover it, but for the refusal of the India Office authorities to allow him the facilities he asked of them Yet in the summer of 1883, only

a few months after MACNAMARA had been refused them by the British authorities KOCH obtained the facilities for his investigation in Egypt which enabled him to isolate his "comma bacillus" from cholera stools. Comment on this sad episode is unnecessary. N. C. MACNAMARA had the misfortune to be too far ahead of his British medical contemporaries, but it is high time justice should be done to his memory by this record of his labours on the most serious of the epidemic diseases of India. The neglect and worse, official discouragement, of pioneer research workers in India of MACNAMARA's day are past; yet over three decades later a somewhat similar incident occurred in India, which is not yet ripe for historical record.

TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE
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DDT AND GAMMEXANE AS RESIDUAL INSECTICIDES AGAINST *ANOPHELES GAMBIAE* IN AFRICAN HOUSES

BY

R C MUIRHEAD-THOMSON, D SC ,
Colonial Medical Research

In previous work in West Africa (MUIRHEAD-THOMSON, 1947 , 1948), a simple and efficient technique was worked out for studying the effects of treating houses with insecticides Those experiments revealed the fact that large numbers of *A gambiae* could escape unharmed from African village houses treated with DDT in kerosene at the rate of up to 250 mg DDT per sq ft

These methods have recently been repeated at Dar-es-Salaam, Tanganyika, to compare the effects of DDT water dispersable powder, and B H C in the form of " Gammexane " water dispersable powder, P 530 A short summary of these findings has already appeared (MUIRHEAD-THOMSON, 1949), the main conclusion being the marked superiority of gammexane over DDT against *A gambiae* It remains to describe more completely the details of technique, numbers of mosquitoes, etc

The domestic habits of the local fresh-water gambiae, salt-water gambiae and *A funestus* will be described in a separate paper

The type of experimental hut used has been described before, but a brief repetition will not be out of place The rectangular huts have mud walls and palm thatch roof, all superimposed on a bamboo framework (Plate I) Actual dimensions were Length, 11 ft , breadth, 7 ft. , height to eaves, 4 ft , height to top of roof, 7 ft Apart from the 1 foot-square window opening, the hut is practically light proof A canvas curtain over the inside and outside of the door admits only the minimum of light on entering or leaving the hut. There is no space between the top of the walls and the roof, the thatch fitting closely all round, but not so closely that hungry mosquitoes can not enter through the innumerable minute cracks and crevices

The detachable window trap cage (Plate II) has already been described Those used in Dar-es-Salaam were constructed on wooden frames, which were

PLATE I.—Experimental Hut with window trap attached.

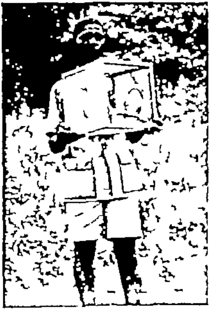

PLATE II.—Detachable Window Trap.

preferable to wire frames which tend to rust and rot the white mosquito gauze The local African collectors soon became proficient at making these to order

In each hut two to four paid Africans slept every night The most satisfactory arrangement was when the Africans actually made the experimental huts their homes In this respect, children aged about 7 to 10 proved most suitable as they were proud of being house owners at such an early age Despite the attraction of such easy money, there is often difficulty in finding reasonable reliable human occupants These difficulties proved insuperable in one locality where huts built in an ideal position to study funestus had to be abandoned because the presence of lions in the neighbourhood upset the Africans At all phases of the experiments constant personal supervision was necessary

For each experiment two identical huts were used When the mosquito population in these huts had reached a high and reasonably steady figure, daily collections were made in hut and window trap to give pre-treatment figures for 2 or 3 weeks One of the huts was then sprayed inside on walls and roof with insecticide, using a " Four Oaks, Kent," knapsack sprayer On the following day the floor was covered with a single large white sheet which fitted close up against the walls, and remained there, apart from daily removal and cleaning, throughout the course of the experiment

The other hut was left untreated as a control

In Dar-es-Salaam there are only 3 or 4 months of the year when conditions are suitable for such experiments, and even the most carefully planned experiment may have to be abandoned because of unexpected falling off in mosquito population

The two experiments described in this paper are the most successful and conclusive carried out As the number of suitable huts was limited, the dosage of each insecticide used was twice that recommended, so that any differences between the two might be emphasized The samples of insecticide were supplied through the courtesy of Mr K S HOCKING, of the Colonial Insecticide Research Unit

DDT DISPERSABLE POWDER

One particularly suitable hut was treated with DDT water dispersable powder (1 lb to 1 gallon of water) at the rate of approximately 400 mg DDT per square foot That is, twice the recommended dose After treatment the hut was visited daily except Sundays Inside the hut search was made for live mosquitoes on walls and roof, and for dead or dying mosquitoes on the floor sheet The window cage was removed and replaced with an empty one , the mosquitoes in the window cage being later transferred to a cage in the laboratory, where they were supplied with raisins, and their mortality noted over the following 48 hours After that time there is an increasingly heavy mortality in ordinary wild caught anopheles, and comparisons are no longer accurate. As collections were made every morning, all anopheles were either blood-fed or unfed, no gorged females being given time to develop their ovaries

TABLE I. TREATMENT OF EXPERIMENTAL HUT WITH DDT DISPERSIBLE POWDER (1 LB. TO 1 GALLON WATER) AT RATE OF APPROXIMATELY 400 MG. DDT PER SQUARE FOOT

NUMBERS OF *Anopheles gambiae* ([illegible]) RECORDED BEFORE AND AFTER TREATMENT COMPARED WITH UNTREATED CONTROL. [illegible]

TREATED HUT

	Weeks before treatment				*Weeks after treatment*				
	3				1	2	3	4	5
Number of collections	3	4	*Number of collections*		5	6	6	3	3
Blood-fed gambiae: Hut	68	148	Inside hut: Blood-fed	Alive	0	0	0	0	[illegible]
				Dead	*0*	*1*	*1*	*0*	[illegible]
Blood-fed gambiae: Window trap	1	4	Inside hut: Unfed	Alive	0	[illegible]	0	[illegible]	[illegible]
Mean daily catch	23	38		Dead	0	1	0	7	1
			Window cage: Blood-fed	Alive	65	118	176	61	31
				Dead	0	0	[illegible]	0	[illegible]
			Window cage: Unfed	Alive	9	31	24	34	3
				Dead	0	0	0	0	[illegible]
			Mean daily catch		*18*	*25*	*33*	*22*	*17*

UNTREATED HUT

	Weeks before treatment.				*Weeks after treatment*				
	3	2			1	2	3	4	5
Number of collections	3	4	Number of collections		3	3	3	3	3
Blood-fed gambiae: Hut	16	31	Total gambiae (hut and window trap)	Blood fed	19	23	6	20	13
Blood-fed gambiae: Window trap	1	0		Unfed	0	0	0	8	0
Mean daily catch		8	Mean daily catch		6	7		8	4

In the untreated control collections were made three times a week The experiment was carried out in an area of pure fresh-water gambiae and funestus, during May and June, 1948 (Table I)

The most striking feature of these results is the great scarcity of dead anopheles on the floor sheet inside the hut, compared with the large numbers of living blood-fed and unfed females taken every day in the window cage During 5 weeks after treatment only two blood-fed gambiae females were found dead inside the hut, while in the same period 449 blood-fed females were taken alive in the window cage The corresponding figures for unfed females were nine and 104

These figures show quite convincingly that blood feeding continues actively inside the treated hut, and that the number of blood-fed females killed inside the hut is only a negligible fraction of those escaping alive from the hut.

Another striking feature, which is not brought out when figures are grouped in weekly totals, is that there is no initial period of protection from biting after the heavy treatment, active blood feeding taking place as early as the first night after treatment, and engorged females escaping alive

In the West African experiments using 5 per cent DDT in kerosene, treatment of houses gave an initial period of protection of 4 or 5 days, during which time there was no biting inside the hut. That effect was shown to be due to the repellent action of the heavy dose of kerosene accompanying the DDT In the present experiment the dispersing agent is water, and there is nothing to repel gambiae from entering and feeding

On comparing the figures from treated and untreated huts, it will be seen that in the control about 6 per cent of all females were unfed, while in the treated hut 20 per cent were unfed. The increase is possibly due to the fact that some hungry females have sufficient contact with treated walls and roof to become irritated with DDT before they have a chance to feed

In experiments of this kind it is difficult to compare accurately the number of mosquitoes biting in treated and untreated huts Some huts consistently record a higher catch of gambiae than others, and it is such huts which are usually selected for treatment In this experiment the mean daily catch of gambiae in the control remained consistently lower than in the treated hut, and the main value of such a control is to indicate the general course of anopheline infestation As far as we can judge from these figures, there is no marked falling off in the numbers of gambiae entering a hut after treatment with DDT

Having established the fact that large numbers of blood-fed gambiae can escape alive from the treated hut, it is now necessary to follow up the subsequent fate of these mosquitoes (Table II)

It will be seen that practically all those blood-fed females which escape from the treated hut are still alive after 24 hours By the end of 48 hours there is a variable mortality which never exceeds 20 per cent Beyond that period a heavy mortality sets in in controls and it is not possible, therefore, to get

accurate comparisons beyond this point. It was noted, however that many of the mosquitoes caught escaping from the treated hut were still alive after 5 days.

The conclusion seems to be that not only do large numbers of blood-fed gambiae escape alive from the heavily treated hut, but that the majority of them also escape unharmed.

It has been suggested that the presence of a distinct window opening in these experimental huts might give irritated mosquitoes unusual opportunities for easy escape such as might not exist in the ordinary African house. But the ordinary African village house is rather a ramshackle affair and while it may have no actual window opening there are numerous holes and gaps, especially between walls and roof through which light can enter. In the experimental hut the 1 foot-square window opening is merely a concentration of all the numerous holes and gaps which normally exist in the African house and which would normally provide easy means of egress to mosquitoes.

When we consider that this experimental hut was treated with DDT dispersable powder at twice the recommended dosage, and that the insecticide was being tried out under ideal conditions where every resting surface was heavily treated in a way that would seldom be possible in the ordinary village

TABLE II. TREATMENT OF EXPERIMENTAL HUT WITH DDT DISPERSABLE POWDER. SUBSEQUENT FATE OF BLOOD-FED GAMBIAE FROM WINDOW CAGES.

Weeks of treatment	2	3	4
Number of blood-fed gambiae tested	70	141	47
alive after 24 hours in laboratory	69	138	47
48	57	116	42
Percentage alive after 48 hours	80	82	89

TABLE III. TREATMENT OF EXPERIMENTAL HUT WITH DDT DISPERSABLE POWDER. EFFECT OF COVERING WINDOW OPENING WITH TRANSPARENT CLOTH SCREEN PREVENTING MOSQUITOES FROM ESCAPING, COMPARED WITH PARALLEL OBSERVATIONS WITH ORDINARY WINDOW TRAP ATTACHED. 6 OBSERVATIONS

		Window trap attached.		Window screened
		Hut.	Window trap	Hut
Gambiae	Blood-fed	6	87	7*
	Unfed	7	35	7*

* Dead on floor sheet of hut.

house, the results suggest that this method does not hold out much hope of controlling *A gambiae* at least

The number of *Anopheles funestus* in these huts was rather low to give conclusive results, and although they are hardly worth tabulating in full they do suggest that the reactions of funestus to DDT may be rather different from those of *A gambiae*

The condensed results are shown in Table IV, figures for mean daily catches being approximate

In the treated hut all the blood-fed funestus were found alive in the window trap, a single one being found dead on the floor sheet in the 5-week period These figures suggest that funestus which feed inside the treated hut are irritated in the same way as gambiae, and are attracted to the window cage

TABLE IV EFFECT OF HOUSE TREATMENT WITH DDT DISPERSABLE POWDER ON *Anopheles funestus* (EXPERIMENT AS IN TABLE I)

		Before treatment (2 weeks)	*After treatment* (5 weeks)
Treated Hut			
Funestus	Total Blood-fed	53	13
	Unfed	0	0
	Mean daily catch	8	0 5
Untreated Hut			
Funestus	Total Blood-fed	9	25
	Unfed	0	0
	Mean daily catch	1	2

However, there seems to be a marked falling off in the numbers of funestus after the hut was treated with DDT, to roughly one-sixteenth of what it was before treatment In the same period the population in the untreated control doubled This suggests that treatment with DDT dispersable powder does prevent many funestus from entering the hut, possibly because they settle longer at the junction of wall and roof before entering, and are irritated by contact with DDT before actually entering the hut.

One would like to repeat the experiment in a more populous funestus area, and also to follow up the fate of those females which do feed in the treated hut and escape by the window

BLE V. TREATMENT OF EXPERIMENTAL HUT WITH GAMMEXANE DISPERSIBLE POWDER, 330 … TE OF APPROXIMATELY 4 MG. GAMMA ISOMER PER SQUARE FOOT

NUMBER OF *Anopheles gambiae* RECORDED BEFORE AND AFTER TREATMENT

TREATED HUT

			Weeks before treatment.			Weeks after treatment.												
			3	2	1	1	2	3	4	5	6		8	9	10	11	12	13
Number of collections			2	3	3	[illegible]	[illegible]	5	[illegible]	[illegible]	4	4	4	5	3	3	3	2
ude ort	Blood-fed	Alive	27	64	312	0	0	0	0	0	0	0	0	0	0	0	0	0
		Dead				74	91	[illegible]	53	70	29	32	33	38	28	[illegible]	[illegible]	[illegible]
	Unfed	Alive				0	0	0	0	0	0	0	0	0	0	0	0	0
		Dead				82	33	16	[illegible]	26	[illegible]	1	0	10	1	4	[illegible]	3
ndow ·age	Blood-fed	Alive				0	0	0	0	0	0	0	0	0	0	0	0	1
		Dead				3	1	7	11	29	3	3	0	[illegible]	[illegible]	[illegible]	0	0
	Unfed	Alive				0	0	0	0	0	0	0	0	0	0	0	0	0
		Dead				0	4	[illegible]	4	4	[illegible]	[illegible]	0	0	0	[illegible]	0	[illegible]

UNTREATED HUT.

Number of collections		2	3	3	3	2	2	3	4	3	2	3	2	1		
Total gambiae	Blood-fed	82	47	56	43	51	1	1	95	39	27	11	15	7		
	Unfed				8	5	0	4	8	5	0	4	0	0		

From the number of dead blood fed females it is evident that feeding still takes place on the occupants of the treated hut, but a high proportion of mosquitoes are killed before they can feed, especially in the first 2 or 3 weeks after treatment.

Among those that feed in the hut there is no indication of that irritation which drives them out of DDT treated houses. The window trap catch remained fairly low after treatment, and all anopheles found there were already dead. Of those escaping from the hut there was no sign, up till 13 weeks after treatment of any surviving.

The continued steady figures for dead anopheles on the floor sheets is proof of continued lethal action for at least 3 months after treatment.

The numbers of funestus were unfortunately too low to give conclusive results, but occasional dead blood-fed females were taken on the floor sheets up till 12 weeks after treatment—the total found being 22

Small numbers of dead blood-fed and unfed *Culex* sp were also taken every week throughout the experiment, and no living culicines were found in the window cage

This experiment shows clearly that gammexane dispersable powder is very much more effective against gambiae than DDT is Even allowing for the fact that the dosage is twice the recommended figure, and that in such experimental huts the insecticide is being tried out under ideal conditions, it appears that gammexane will be a residual insecticide of real value against *Anopheles gambiae* at least

In previous work in Sierra Leone, DAVIDSON (1947) obtained encouraging results in malaria control by house treatment with earlier forms of gammexane Unfortunately, his observations were not continued long enough to be quite conclusive, and there was nothing to indicate whether the absence of anopheles from treated houses was due to their being actually killed, or whether they were merely being driven out as with DDT Furthermore, the earlier forms of gammexane were not as effective as the present P 530 (P 520 is the most recent form)

More recently, however, DAVIDSON (1949) has tested out P 530 in the Belgian Congo, paying particular attention to the reactions of mosquitoes in treated houses His results show a very marked reduction in number of mosquitoes caught, significant numbers of mosquitoes not appearing in window traps till the 15th or 16th week after treatment His results so far mainly apply to *Anopheles moucheti*, but they form a valuable link up with the present work on gambiae in East Africa

SUMMARY

(1) Experimental mud and thatch huts, with window traps attached, have been used to compare the effect of house treatment with residual insecticides against *Anopheles gambiae* in East Africa

(2) In huts treated with DDT water dispersable powder at the rate of 400 mg DDT per square foot (twice the normal dose), large numbers of gambiae can feed in the treated hut and escape alive

(3) Of those that leave the treated hut at least 98 per cent are alive after 24 hours, and at least 80 per cent after 48 hours The number of *A gambiae* found dead inside the treated hut is only about 1 per cent of those that escape alive after feeding Attempts to increase the kill by screening window openings have only met with limited success

(4) Similar huts treated with B H C in the form of " gammexane " dispersable powder, P 530 at the rate of 24 mg gamma isomer per square foot, have proved completely lethal to all gambiae entering for at least 13 weeks after

treatment. During that time there is no indication of any gambiae escaping unharmed from the hut. The number of dead blood-fed and unfed gambiae found regularly on the floor sheets is proof of continued lethal action for at least 3 months after treatment.

(5) The superiority of gammexane over DDT in control of *A. gambiae* is so clearly marked that it is the obvious insecticide on which to concentrate.

REFERENCES.

Davidson, G. (1947). *Ann. trop. Med. Parasit.*, 41, 178.
——— (1949). *Brit. med. J.* 1, 101.
Garrett S. (1948). *Trans. R. Soc. trop. Med. Hyg.* 42, 295.
Muirhead-Thomson R. C. (1947). *Bull. ent. Res.*, 38, 449.
——— (1948). *Ibid.*, 39, 527.
——— (1949). *Nature*, 163, 109.

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AN INVESTIGATION OF RHEUMATIC FEVER IN FIJI *

BY

GODFREY BARNES, M B , D T M & H ,
Government Pathologist, Fiji

The objects of the present investigation were to determine what evidence exists for the diagnosis of rheumatic fever in the Fijians and Indians in Fiji, and, if its presence is accepted, to obtain some idea of its incidence

The authorities on diseases in the tropics continue to dismiss rheumatic fever as of little importance in those parts of the world In the latest edition of his text-book MANSON-BAHR (1945) states that rheumatic fever and rheumatic valvular heart disease are infrequent in native races According to the American authority, Stitt's *Tropical Diseases* (STRONG, 1942), records suggest that rheumatic fever is extremely rare in natives in the tropics and that rheumatic valvular disease also is rare Both authorities quote reports by various writers of considerable numbers of cases of rheumatic fever and rheumatic carditis in various parts of the tropics, *e g* , Ceylon and the Gold Coast, but express doubt as to the reliability of diagnosis MANSON-BAHR asserts that valvular heart disease in natives is usually syphilitic and expresses the belief that some cases diagnosed as rheumatic fever may be gonococcal arthritis ROGERS (1942) is very categorical in expressing his view that rheumatic fever and rheumatic heart disease are of " remarkable infrequency " in Bengal, and he suggests that this infrequency

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For the photographic work I am indebted to the Laboratory Superintendent, Mr J E PERY-JOHNSTON

holds for all India. He also attributes recorded cases in India to mistaken diagnosis. Only one case, an Anglo-Indian, occurred in 4,800 autopsies in 37 years. Several thousand specimens in the pathology museum in Calcutta include only one described as rheumatic endocarditis in an Indian subject, and ROGERS, after examining this specimen, thinks that even this one may be non-rheumatic.

A prejudice may exist against the acceptance of rheumatic fever in the tropics as a result of the views on aetiology generally held for the disease as it occurs in Europe. The Medical Research Council's publication *Social Conditions and Acute Rheumatism* (1927), reporting comprehensive studies of possible aetiological factors, shows that none is definitely known to be operative except a degree of poverty. Yet it is usual for confident opinions to be expressed which by implication make the tropics a most unlikely place in which to come across this disease. LEWIS (1943) writes: "The disease is one of cold and damp countries of the northern hemisphere and is found particularly among the over-crowded and poorer families of towns. It appears especially in the winter months. In the *British Encyclopaedia of Medical Practice* (1938) we read: "Acute rheumatism is a disease of temperate climates. . . . It is a winter disease. . . . It is a disease of urban rather than rural districts, and of industrial towns in particular."

The scepticism evinced by the leaders in tropical medicine to an appreciable occurrence of rheumatic fever in the countries with which they are concerned demands that statements to the contrary by lesser men must, in order to receive serious consideration, be supported by evidence as complete as the nature of the subject will allow. As it is, little work of a convincing nature appears to have been done, while much of the available information is hardly calculated to inspire confidence in the medical sources which must accept responsibility for it. One can readily imagine the contempt for Colonial statistics which must result from reading the figures for rheumatic fever published in "A Geography of Disease" (MCKINLEY, 1935) where the information is derived from Annual Medical and Health Reports of the Colonies and from officially-compiled replies to a questionnaire circulated by the author. First of all there is the pathetic confusion between "rheumatic fever" and "acute rheumatism." For example Uganda and Tanganyika each returns a figure of approximately 300 cases against "rheumatic fever" and makes reference to a footnote which reads "acute rheumatism," as though to imply that in these countries the disease takes a form known as "acute rheumatism." Nigeria, on the other hand, gives "rheumatic fever" as rare, but comments that there were 3,500 cases of "acute rheumatism" as though to say that the two diseases are quite different but liable to terminological confusion. Fiji, in this publication, is guilty of an inconsistency peculiar to herself. In answer to the questionnaire it is stated that there was an average of 91 admissions to hospital with rheumatic fever over 3 years, but an adjacent list of diseases taken from an Annual Medical and Health Report, presumably for one of these 3 years, shows only six cases for that year.

In contrast to all this scepticism and confusion there is a certain amount of research being done in tropical countries from which the true position with regard to rheumatic disease in the tropics will gradually evolve. An example of this is the account by KUTUMBIAH (1940) of the histological lesions in the hearts of fatal rheumatic fever patients in South India, illustrated with photomicrographs which demonstrate the conclusive presence of Aschoff nodules.

CRITERIA FOR DIAGNOSIS

(1) *Aschoff nodules*

This form of cellular inflammatory reaction has been produced experimentally in rabbits as a manifestation of serum hypersensitivity (McKEOWN, 1947), and has been found in association with subacute bacterial endocarditis (MACILWAINE, 1947), but these discoveries have been used to elucidate the nature and the complications respectively of rheumatic fever, and, as far as I am aware, no one doubts the specificity of the nodules for this disease. Histological demonstration of typical Aschoff nodules has been accepted by me in this investigation as absolute proof of rheumatic fever.

(2) *Rheumatic vegetations*

These are very characteristic in appearance and, if for any reason a histological investigation was impossible, would alone constitute at least a probability of rheumatic fever, which would become proof if supported by a clinical history.

(3) *Polyarthritis*

Migratory acute or subacute periarticular arthritis, affecting the joints ranging in size from the metacarpophalangeal to the shoulder can be very characteristic. Unfortunately, as will be seen from the case notes which are entered as an appendix to this report, a clinical picture which in general conforms to this description is not uncommonly complicated by the occurrence of a synovial effusion, or unusual joints such as the interphalangeal or hip may be most complained of, or the response to salicylates may be slow, and so on, so that doubt arises as to the diagnosis. In the present investigation I have recorded as " doubtful, probable " those cases in children or young adults where the polyarthritis, or a reliable history of it, follows for the most part the accepted pattern and where no laboratory evidence can be found for alternative diagnoses, such as gonococcal, dysenteric, enteric, brucellosis, or meningococcal arthritis. I have labelled " clinically proved " those cases which, in addition to the above requirements, provide evidence either of active carditis or of chronic endocarditis.

(4) *Active carditis*

Active carditis as an apparently isolated condition, when other causes of pericarditis and also ulcerative and subacute bacterial endocarditis have been

excluded, w uld justify a diagnosis of rheumatic fever Such cases are well known to be common in children in English practice and, according to KUTUMBIAH (1941) are even more a feature of juvenile rheumatic fever in South India, but the patients with active carditis whom I have encountered in Fiji have all had polyarthritis, either at the time or shortly before

(5) *Chorea*

This manifestation of the disease was not seen by me during the course of my survey although one or two cases are returned for most years in the annual report of the Colonial War Memorial Hospital. Chorea, as with a characteristic polyarthritis, would justify a diagnosis of doubtful probable " or " clinically proved " rheumatic fever according to what additional evidence could be derived from the history and the clinical findings.

(6) *Chronic endocarditis*

Mitral stenosis discovered during life at any age or postmortem constitutes a probability of rheumatic fever in the past. It is just precluded from recognition as proof by its reported occurrence rarely as a congenital defect and by the possibility that it may result from the calcification of the mitral valve occurring not infrequently according t EAST and BAIN (1948) as a degenerative change in elderly people. Judging from the apparent frequency and severity of atheroma in early middle-age degenerative changes in the valves may take place in Fijians and Indians at a lower age level than among Europeans in temperate climates in the same way that, according to KING (1942), negroes in America show cardiovascular senescence at 40 comparable to that of a white man of 50. Congenital mitral stenosis is generally accepted to be extremely rare only one case of congenital mitral stenosis and seven of atresia of the mitral nd ortic valves were found in nearly 28 000 autopsies in America and Canada (KING, 1942) but there is no certainty that it is equally rare in Fiji.

Needless to say the finding of Aschoff nodules or the scars resulting from them in a heart with mitral stenosis would prove that rheumatic fever had existed but I do not think that, in the present state of our knowledge failure to find traces f Aschoff nodules in these cases should be held to prove non-rheumatic cause of the stenosis.

One will sometimes meet with aortic regurgitation n a child or in young adult where syphilis or other causative disease can be excluded, and in whom evidence of associated mitral stenosis is indefinite. Such a case (A.27) was encountered by me during thi investigation and with confirmatory associated findings was diagnosed unhesitatingly as clinically proved heumatic fever

RESULTS OF THE INVESTIGATION

Throughout 1948 I endeavoured to see every patient admitted to the Colonial War Memorial Hospital Suva, who might show evidence of past or

present rheumatic fever To this end the medical officers of the hospital referred to me

(*a*) All patients with polyarthritis which was not clearly attributable to some other cause, and

(*b*) All heart cases, acute or chronic, in patients up to the age of 35

The material presented itself clinically in one of three forms

(*a*) As possible rheumatic fever, *i e*, the active disease in one or other of its manifestations,

(*b*) As chronic endocarditis, and

(*c*) As congestive heart failure

In accordance with the criteria for diagnosis outlined earlier a classification was prepared into which all cases seen would fit, as follows

(*a*) Proved rheumatic fever

(*b*) Clinically proved rheumatic fever

(*c*) Doubtful, probable rheumatic fever

(*d*) Doubtful, improbable rheumatic fever

(*e*) Proved non-rheumatic

The Table shows the distribution of the cases in this classification

TABLE.

CLASSIFICATION OF 40 CASES INVESTIGATED FOR RHEUMATIC FEVER

	Proved		Clinically proved.		Doubtful, probable		Doubtful, improbable		Proved non-rheumatic		Total
	Fij	Ind.	Fij	Ind.	Fij	Ind	Fij	Ind.	Fij	Ind	
Rheumatic fever		1		7		11	2	8			
Chronic endocarditis due to rheumatic fever					2	1					
CHF due to chronic rheumatic endocarditis				3		2	2	1			
Totals	0	1	0	10	2	14	4	9	0	0	40
	Fij 2, Ind. 25 } 27										

Of a total of 40 subjects 27 were proved, clinically proved, or probable—0 7 per cent of the native admissions to the hospital for the year But there was a pronounced racial difference Twenty-five were Indians—1 2 per cent of the total Indian admissions to hospital, and only two were Fijians—0 2 per cent of the total Fijian admissions to hospital The corresponding figure for the

Colombo General Hospital (FERNANDO, 1939) is 2·2 per cent., and for a New York hospital (SWIFT and MCEWEN, 1938) 5·9 per cent. SWIFT (1931) states that acute rheumatic polyarthritis alone is generally regarded as responsible for 3 to 7 per cent. of all admissions to large general hospitals in the United States and Europe. Thus rheumatic fever appears to be about twice as common in Ceylon and *more than three times as common in Europe and North America* as among Indians in Fiji.

Of children aged 6 to 10 inclusive, six, all Indians, were diagnosed as cases of rheumatic fever either "clinically proved" or "doubtful, probable" and *they constitute 8 per cent. of all Indians in this age group who were admitted* to the children's ward during the year. I have not been able to find hospital figures from other countries for in-patients of this age-group with which to compare my figures. Among out-patients, however, STILL (1927) in London *found 13 per cent. of children aged from 6 to 10 of the hospital class*, but less than 1 per cent. of children of the same age in a good-class practice, with evidence of rheumatic infection. It would seem then that the incidence of rheumatic fever among Indians in Fiji is higher than that among well-to-do people in England and lower than that among the poor.

The Indian cases investigated comprise one proved rheumatic fever, 10 clinically proved cases, 14 probable cases and nine improbable cases. Only one case (No. A2, see appendix) was proved by postmortem findings and histology (Fig. 1). This was an Indian girl aged 11 who showed no detectable stenosis as evidence of any earlier attack and who died after a short and stormy illness in hospital with auricular fibrillation, congestive heart failure and fever, a form of the disease which I believe has been referred to as "malignant rheumatic carditis." The cases classed as "probable" and those classed as "improbable" are listed in the appendix with a summary of the evidence for and against the diagnosis, but the principles involved in accepting or rejecting the diagnosis are as outlined earlier. Fig. 2 (PM 43/48) shows the heart of an Indian male (aged 45 and therefore not included among the cases analysed in this investigation) with mitral and aortic stenosis typical of rheumatic fever although sections have not revealed Aschoff nodules. A case of this nature falls under the heading "doubtful, probable." Some of the "doubtful, improbable" cases would certainly have been recorded as rheumatic fever in the ordinary way of hospital and private practice, but the evidence available provides less than a probability of the disease and to accept them could only serve to justify the doubts as to the reliability of diagnosis which have been expressed by MANSON-BAHR and ROGERS to which reference was made at the outset.

As for rheumatic fever in the Fijians, my investigations have disclosed, or at least confirmed, two important facts: (i) that the disease does definitely occur among them, and (ii) that it is considerably more rare in Fijians than in Indians. During the 12 months' period devoted to the analysis of cases, acceptable Fijian material was limited to two "doubtful, probable" cases, one a fatal one of

FIG. 1.—An Aschoff nodule in the myocardium of an Indian, female, aged 11

FIG. 2.—Aortic and mitral stenosis: black pointer in mitral valve. (Upper black area is site of tissue removed for histology.) Indian, male, aged 45

FIG. 3 —Mitral stenosis and calcification in valve and in wall of left auricle (The hole above the glass rod and the incision into the lower margin of the valve were made to obtain tissue for histology) Tylan, female aged 30

FIG. 4 —Rheumatic vegetations on all aortic and mitral cusps. Fynan, female aged 17

FIG. 5 —An Aschoff nodule in the myocardium of Tylan, female aged 17

cerebral embolism due to gross mitral stenosis (Fig 3) (PM 85/48), and the other a young apparently fit Fijian police recruit with typical clinical mitral stenosis confirmed to some extent by ECG and X-ray In the museum here further evidence of this sort is supplied by a heart showing gross mitral stenosis (PM 34/43) removed at autopsy on a Fijian woman of 40 who died in 1943 as a result of abortion and uterine haemorrhage By the time that the period allotted to the investigation had come to an end, no more definite indication of the occurrence of rheumatic fever among the Fijians had been encountered than these cases of mitral stenosis In January of the present year, however, a Fijian girl of 17 died in hospital after a few days' illness with fever and pericarditis, and at postmortem she was found to have a typical acute rheumatic endocarditis of mitral, aortic, and tricuspid valves (Fig 4) (PM 19/49), and sections revealed many Aschoff nodules (Fig 5) This is the first, and so far the only, absolutely proved case of rheumatic fever in a Fijian

The Fijian cases investigated included two which were classed as "doubtful, improbable," and the evidence for and against the diagnosis is summarized in the appendix to this report (cases No B 1, 2) The pitfalls of diagnosis are vividly demonstrated by a patient who came under hospital treatment this year, and so does not come into the present analysis He was a Fijian male of 31 who presented severe congestive heart failure together with a pericardial rub, and rheumatic carditis was diagnosed A postmortem examination, however, revealed an enormously hypertrophied heart and not, as had been thought, simply a grossly dilated one, chronic glomerulonephritis, and a terminal fibrinous pericarditis, but no valvular disease whatever (PM 33/49)

The relative infrequency of rheumatic fever in Fijians as compared with Indians—the incidence among Indian admissions to hospital was six times that among Fijian admissions—should be of some use in any attempts which may be made in Fiji to elucidate the baffling aetiology of this disease

SUMMARY

The criteria for the certain and probable diagnosis of rheumatic fever and its sequelae have been outlined These criteria were applied to all cases possibly suffering from this disease which were admitted to the wards of the Colonial War Memorial Hospital, Suva, Fiji, during the year 1948

Rheumatic fever has been proved with certainty in both the Fijian race and among the Indians born and living in Fiji

Indian cases, proved absolutely, "clinically proved" and "doubtful, probable," were 1 2 per cent. of total Indian admissions (2,067), Fijian cases 0 2 per cent of total Fijian admissions (1,162), suggesting an incidence six times as great among the Indians of Fiji as in the indigenous Fijian race

In the age group 6 to 10, when, in Europe and America, the disease usually

first attacks and has its highest incidence, only Indian cases came under observation, and these constituted 8 per cent. of the total Indian admissions (73) for these ages.

REFERENCES.

British Encyclopaedia of Medical Practice (1938). 10 640 London Butterworth & Co., Ltd.

EAST T & BAIN C. (1948) *Recent Advances in Cardiology* 4th Ed., 57 London J & A. Churchill, Ltd.

FERNANDO P E. (1939). *Quart. J. Med.*, 8, 261 quoted by the *British Encyclopaedia of Medical Practice Surveys and Abstracts.* (1940). 510 London Butterworth & Co. Ltd.

KING, J T & BRAMWELL, C. (1942). *The Principles and Practice of Cardiology* 283 and 293. London : Humphrey Milford.

KUTUMBIAH, P (1940). *Indian J med. Res.*, 27 631

——— (1941) *Indian J Pediat.* quoted by *The Medical Annual* (1943). 304 Bristol John Wright and Sons, Ltd.

LEWIS, T (1943). *Diseases of the Heart* 3rd Ed., 195 London : Macmillan & Co., Ltd.

MACILWAINE, Y (1947). *J Path. Bact.* 59 557

MCKEOWN E. F (1947). *Ibid.*, 59 547

MCKINLEY E. B. (1935) *A Geography of Disease* Washington The George Washington University Press.

MANSON-BAHR, P H. (1945). *Manson Tropical Diseases* 12th Ed., 38 London Cassell & Co., Ltd.

MEDICAL RESEARCH COUNCIL REPORT (1927). *Social Conditions and Acute Rheumatism.* London H.M. Stationery Office.

ROGERS, L. & MEGAW J W D (1942). *Tropical Diseases* 4th Ed., 497 London J & A Churchill, Ltd.

STILL, G F (1927) Introduction to *Social Conditions and Acute Rheumatism* Med. Res. Coun. Rep 7 London H.M. Stationery Office.

STRONG R. P (1942). *Stitt' Diagnosis Prevention and Treatment of Tropical Diseases,* 6th Ed., 1803 London H. K. Lewis & Co., Ltd.

SWIFT H. F (1931) *Nelson New Loose-Leaf Medicine* 1 402. New York Thomas Nelson & Sons.

——— & MCEWEN C (1935) *Oxford Medicine* 5 15 New York Oxford University Press.

APPENDIX.

CASE NOTES.

GROUP A. INDIANS. RHEUMATIC FEVER ?

Case No. 1 Fem. age 11 *For the diagnosis* Acute polyarthritis of typical intravescence character distribution, and typically migratory Response to salicylates. Mitral systolic murmur develops in convalescence. Blood culture, faeces culture for typhoid and dysentery and agglutination tests for brucellosis or recent typhoid all negative.

Against the diagnosis Joints affected include two interphalangeal and sternoclavicular No nodules. PR interval of electrocardiogram (ECG) not prolonged. Returned with apyrexial pain in knees with coarse crepitus. *Doubtful, probable*

Case No. 2. Fem., age 11 *For* Auricular fibrillation, congestive heart failure (CHF), mild bilateral arthritis of knees (with effusion) pyrexia. Postmortem (PM), typical vegetations on mitral and tricuspid valves Aschoff nodules in myocardium. (Fig 1) *Proved.*

Case No. 3 Fem., age 14 *For* History of typical polyarthritis. Seen with evidence of acute carditis. Re-examination 1 year later revealed mitral stenosis, confirmed by ECG and teleradiogram (TRG) *Clinically proved*

Case No 4 Male, age 9 *For* History of typical polyarthritis Evidence of acute carditis Classical aortic regurgitation confirmed 8 months later Kahn negative *Clinically proved*

Case No 5 Male, age 13 *For* Acute polyarthritis of typical character and distribution Evidence of transient carditis (mitral systolic murmur) Response to salicylates Blood, stool, and urine culture negative for pathogens, typhoid and brucellosis agglutination tests negative

Against Onset of polyarthritis said to have been coincident in all affected joints Abdominal pain and tenderness, complaint of dysuria, some doubtful microscopic evidence of pyelitis—epithelial cells ++++ at one examination, pus cells ++ at another No nodules PR interval of ECG normal One year later has kept well, full activities, heart normal, urine normal *Doubtful, probable*

Case No 6 Male, age 34 *For* History of typically migratory acute polyarthritis and of response to salicylates Cultures of blood, stools, urine, no pathogens Typhoid and brucella agglutination negative

Against History of onset in hips No history of previous arthritis or sore throats Tenderness under heels (history of recurrent sores here following injuries) Present fever does not respond satisfactorily to salicylates The only arthritis seen by me (third day of illness) a subacute synovitis of a knee Catheter urine albumin +, pus +++, epithelial cells ++++ Prostatic smears pus +, mixed infection ++++ (no gonococci) Therapeutic response to sulphapyridine No cardiac abnormality No further illness in follow-up for 5 months after discharge *Doubtful, improbable*

Case No 7 Male, age 24 *For* Acute polyarthritis of typical ingravescence and character and typically migratory Blood and stool cultures negative, typhoid and brucella agglutination negative Synovial fluid from a knee sterile, no cells No response to sulphadiazine or penicillin

Against Accent on metacarpophalangeal and metatarsophalangeal joints, and proximal interphalangeal joints of hands and feet, and pain in cervical vertebrae No previous arthritis or sore throats Severely ill for 12 weeks without good response to salicylates A temporary soft mitral systolic murmur the only cardiac pathology Attack of ? pyelitis in fourth week (dysuria, albumin ++++, RBC ++++, granular casts +, pus + *B coli* isolated in some cultures, good response to alkalis) Prostatic smears, mixed infection, no gonococci ECG, only sinus tachycardia After recovery no relapse or cardiac pathology over follow-up of 1 year *Doubtful, improbable*

Case No 8 Male, age 15 *For* Acute polyarthritis of typical ingravescence and typically migratory

Against In addition to the usual joints the metacarpophalangeal, metatarsophalangeal, interphalangeal, sternoclavicular, ? laryngeal, and ? cervical vertebral joints persistently affected Character, although generally typical, included synovitis of knees Right knee fluid cloudy, polymorphonuclears, sterile Blood culture, *S aureus* Inconclusive response to salicylates, arthritis waxing and waning over 6 weeks Cardiac pathology, only transient enlargement and faint mitral systolic murmur At 1 month and at 1 year after discharge, no recurrence, good health, heart normal, Kahn + *Doubtful, improbable*

Case No 9 Male, age 40 *For* History of typical polyarthritis

Against Several years' recurrent arthritis in metatarsophalangeal joints of big toes, ankles and knees only History of gonorrhoea in past Only left first metatarsophalangeal and knee joints affected while under treatment Prostatic smear, mixed infection, pus cells +++ Urine, albumin +, *B coli* only in cultures Steady improvement without treatment and later with sulphathiozole and without salicylates Inconstant mitral systolic murmur *Doubtful, improbable*

Case No 10 Male, age 6 *For* Typical acute polyarthritis Response to salicylates Temporary mitral systolic murmur

Against Heart normal during 4 weeks preceding discharge *Doubtful, probable*

Case No. 11 Male, age 6. *For* Typical acute polyarthritis Response to salicylates mitral systolic murmur of varying character often high pitched and musical persisting into convalescence *Clinically proved.*

Case No. 12. Fem., age 18. *For* History of polyarthritis of typical distribution. ESR remained at 14 to [illegible] mm. per 1 hour (Wintrobe) during the [illegible] months of observation. A knee painful [illegible] years ago. Blood stool and urine cultures and typhoid and brucella agglutination negative

Against No signs of arthritis, only symptoms of joint discomfort, when first seen [illegible] weeks after onset. Temperature did not rise above 100 F Only previous relevant history swollen and painful knee for 3 weeks 2 years ago. Heart clinically and ECG normal. *Doubtful probable*

Case No. 13 Fem. age 14 *For* History of acute polyarthritis of typical distribution, responding readily to medicine—? salicylates. Seen 10 days after onset with slight irregular fever for next fortnight. Loud mitral systolic murmur unchanged into convalescence during 4 months follow-up Left ventricle and left auricle hypertrophied—clinically TRG and ECG Diminished cardiac reserve Blood cultures negative Kahn negative

Against No presystolic mitral murmur or aortic murmur Admitted with urgent dyspnoea and cyanosis of sudden onset while convalescing from polyarthritis, and murmur at that time had high-pitched musical quality suggesting perforation of [illegible] cusp. *Doubtful probable*

Case No. 14 Fem age 8. *For* History of 1 week fever Mitral murmur

Against No history of joint pains, sore throat, or heart failure Fever accompanied by cough. TRG and ECG normal. Mitral murmur followed up for 3½ months and diagnosed as cardiorespiratory Innocence confirmed by physician-specialist (Dr P F C. MANSON BAHR) *Doubtful improbable*

Case No 15 Fem., age 22. *For* Acute polyarthritis of typical distribution and character with history of typical onset and that it was migratory History of similar attacks going back into childhood Hospital record of similar attack 3½ years before with mitral stenosis. Mitral systolic murmur. Clinical and ECG evidence of auricular hypertrophy Readmitted 8 months later with severe CHF No aortic disease Kahn negative *Clinically proved*

Case No 16 Male age 7 *For* History of acute polyarthritis of typical distribution and character during the 3 weeks prior to observation and mild recurrences while under my care Response to salicylates. Mitral systolic murmur persisting throughout observation period of 3 to 4 months ECG some evidence of mitral stenosis (inverted P waves).

Against Heart clinically not definitely abnormal TRG normal. ECG within normal limits *Doubtful probable*

Case No. 17 Male age 35 *For* Acute polyarthritis with history of typical migratory recurrence and with typical distribution and mostly of typical character Response to salicylates. Transient mitral systolic murmur No pathogens in cultures of blood, prostatic exudate joint effusion faeces and urine Negative typhoid and brucella agglutination. No amoebae or cysts in stools

Against No throat or previous joint trouble Dysentery three times in last 3 years. Gonorrhoea 15 to 16 years ago (no relapses). Prostatic smears show [illegible] mixed infection, [illegible] few pus cells. Urine at first showing albumin + pus cells [illegible] leucocyte casts RBC hyaline and granular casts gradually improved to RBC pus cells + only on discharge Knee effusion, appearance of thin pus, an occasional extracellular gram-negative coccus. Heart normal after exercise etc., [illegible] months after onset. *Doubtful improbable*

Case No. 18 Male age 10 *For* History of acute polyarthritis during [illegible] weeks prior admission, one typical joint characteristically affected at first examination, and subsequent fleeting discomfort in various joints. Response to salicylates. Mitral systolic murmur loud at first becoming fainter and absent in sitting position during convalescence ECG

shows auricular extrasystoles (and sinus tachycardia) No pathogens in cultures of blood, faeces and urine, typhoid and brucella agglutination negative

Against No mitral presystolic murmur ECG, no prolongation of PR Kahn + *Doubtful, probable*

Case No 19 Male, age 14 *For* Polyarthritis of typical distribution Response to salicylates

Against Synovitis of knees, aspirated fluid contains pus cells and extracellular staphylococci, culture grows *S aureus* Blood cultures, *S aureus*, strongly haemolytic strain (The patient's condition was nevertheless never alarming, and rapidly improved, temperature fell to normal on second day with no other therapy than salicylates) Urine, albumin +, pus cells +++ RBC and epithelial cells +, no casts, cultures sterile, subsequent urine examinations normal Heart, normal clinically and normal ECG during fever *Doubtful, improbable*

Case No 20 Fem, age 15 *For* History of typically migratory acute polyarthritis for 2 months before admission, history of typical distribution, character observed to be typical Response to salicylates Mitral systolic murmur, which had disappeared 5 months after admission No pathogens in cultures of blood, faeces, urine, typhoid and brucella agglutination negative

Against No diastolic murmur developed ECG and TRG normal *Doubtful, probable*

Case No 21 Male, age 10 *For* Acute arthritis of knees of typical character Hospital record of typical polyarthritis 1½ years ago Response to salicylates Mitral stenosis clinically, TRG and ECG Readmitted 3½ months after discharge suffering from CHF *Clinically proved*

Case No 22 Male, age 18 *For* Acute arthritis of knees and an ankle No pathogens in cultures of blood, urine, faeces, joint fluid, prostatic exudate, no amoebae or cysts in stools, typhoid and brucella agglutination negative

Against No previous arthritis, sore throats Arthritis in knees predominantly a synovitis, little discomfort Improvement under salicylates slow although progressive History of dysentery 10 years ago and 1 month ago Kahn + (reverting to negative without antisyphilitic treatment, father Kahn +, mother Kahn doubtful Had seven injections of NAB over 7 months before admission for cracked heels) Heart normal No recurrences over 3 months' follow-up *Doubtful, improbable*

Case No 23 Male, age 7 *For* History of acute polyarthritis of typical distribution 2 to 3 months ago, and of fleeting joint pains over last 2 to 3 years Admitted for ? precordial discomfort, ? general ill-health Evidence of active carditis in mild pyrexia, raised BSR, tachycardia Persistent loud mitral systolic murmur, variable and indefinite mitral diastolic murmur, assentuated second pulmonary sound (P2) TRG suggests some enlargement of conus and left auricle

Against No presystolic or definite mid-diastolic murmur Kahn ++ on two tests (mother Kahn negative, father deceased) *Doubtful, probable*

Case No 24 Male, age 11 *For* Acute polyarthritis of typical distribution and character and history of typical ingravescence Response to salicylates History of similar illnesses 6 months and 1 to 2 years ago Blood, stool and urine cultures, no pathogens, no amoebae or cysts in faeces Widal result attributed to inoculations, brucella agglutination negative

Against Heart normal clinically, TRG and ECG History of dysentery 2 years ago and a few weeks ago Urine, pus cells +, RBC −, epithelial cells +−++ *Doubtful, probable*

Case No 25 Male, age 19 *For* Typical acute polyarthritis History of similar illness 5 years ago, and present illness has lasted, with two intermissions under treatment, for 4 months Two months after present investigation readmitted to hospital with typical migratory polyarthritis and findings as before Response to salicylates Mitral systolic

murmur persisting over the 4 months of total observation period, becoming rougher towards end of the period. No pathogens in cultures f urine prostatic exudate stools, blood. Kahn negative. Agglutination tests for typhoid and brucella negativ

Against Grating of both sternoclavicular joints. Gingivitis and foetor oris. TRG and ECG normal. N diastolic murmu *Doubtful probabl*

Case No. 26. Fem., age 12. *For* Typical acute polyarthritis. Response to salicy lates. History of imilar illnesses 1 year and ? years ago. Mitral systolic murmur constant, accentuated P2. TRG shows some enlargement to right and prominence in region of pulmonary conus. N pathogens i cultures of blood, urine faeces. Typhoid and brucella agglutination negative.

Against No diastolic murmur ECG shows tachycardia only N recurrences in 2½ months following discharge heart unchanged, TRG as before *Clinically proved.*

Case No. 27 Male, age 10 *For* History of migratory polyarthritis in an elbow and feet during 6 days before admission. While under treatment relapse of arthritis of typical character in wrist and shoulder ½ months after discharge has aortic and mitral diastolic murmurs—clinical aortic regurgitation with some confirmatory evidence from TRG and ECG Negativ cultures from blood, joint fluid, faeces, urine *Clinically proved*

GROUP B. FIJI NS. RHEUMATIC FEVER ?

Case No. 1 Fem., age 18 *For* History of polyarthritis or arthralgia in fingers knees and lbows. On first examination ? week after onset discomfort in right elbow right shoulder and right fingers. Similar illness 1 yea ago Sore throats once or twice yearly over last few years.

Against Onset said to hav been accompanied by diarrhoea on both occasions Onset said to have followed eating fish and others who ate the fish to hav been affected similarly t less degree on both occasions. Right elbow injured in childhood fall, X-ray show small separated piece of bone. Steady improvement without drugs except per sistent discomfort in right lbow H art normal. *Doubtful, improbabl*

Case No. .. Fem., age 20 *For* Typical acute polyarthritis. Response to salicylates. Mitral systolic murmur developing during illness and persisting into convalescence.

Against Acute glomerulo-tubular nephritis indicated by albumin + − −, granular casts − − RBC + + pus cells + − + − clearing after 3 weeks. Blood rea 80 mg per 100 ml. and B.P 140/90 becoming normal with normal urine ECG and TRC normal. *Doubtful improbable*

Not an a ase f proved rheumatic fever met n th subsequent to period of collection f data for this investigation Fem., age 17 Acut dry pericarditis and acute CHF fatal after day in hospital, history of 1 day illness before admission and no previous illnesses PM, fibrinous pericarditis, firm pericardial and mediastino-pericardial adhesions typical rheumatic excrescences on aortic, mitral, and tricuspid alves in that order of magnitude no stenoses right and left cardiac dilatation (Fig 4) Histologically typical Aschoff nodules sections from mitral cusp, myocardium of left entricle (Fig. 5) papillary muscle of left entricle and MacCallum patch. I addition, these and other areas of the heart *e.g* pericardium, where no well-defined Aschoff nodules were recognized, showed intense diffuse infiltration with lymphocytes, plasma cells, large mononuclears and polymorphonuclears

Note an suspected use proved non-rheumatic met with subsequent to period of collection of data for the investigation Male age 31 CHF with dry pericarditis and mitral systolic and ? diastolic murmurs, diagnosis of rheumatic carditis made by medical attendant. PM, chroni glomerulo-nephritis, gros left entricular hypertrophy CHF dry pericarditis No naked-ey or histological evidence of endocarditis or myocarditis

GROUP C INDIA N CONGESTIVE HEART FAILURE DUE TO CHRONIC RHEUMATIC ENDOCARDITIS ?

Case No. 1 Male age 11 *For* CHF (normal rhythm), mitral mid-diastolic, presystolic, and systolic murmurs. History of acute polyarthritis 3 years and 1½ years

ago History of CHF with hospital records 1 year and again 11 months ago *Clinically proved*

Case No 2 Fem, age 22 *For* Auricular fibrillation (slow), mild CHF, mitral stenosis

Against No history of arthritis or sore throats One year of thoracic and abdominal discomfort the only history *Doubtful, probable*

Case No 3 Male, age 12 *For* Severe CHF, aortic regurgitation and mitral stenosis diagnosed on clinical and ECG evidence

Against No history of arthritis, sore throats or other illness before present illness began 1 month ago No evidence of active carditis beyond moderately raised BSR *Clinically proved*

Case No 4 Fem, age 12 *For* Severe CHF, fatal after ? 3 months of illness

Against Aetiology obscure during life and postmortem, the dilated heart showing no endocarditis, acute or chronic, and histologically oedema and inflammatory infiltration of epicardium and to a slight extent of the fibrous septa of the myocardium, the inflammatory cells largely lymphocytes with a few plasma cells and they have a perivascular distribution, no Aschoff nodules *Doubtful, improbable*

Case No 5 Fem, age 10 *For* Severe CHF, readmitted twice more with severe CHF during 12 months subsequent to this investigation Mitral stenosis, with confirmatory TRG and ECG Aortic regurgitation diagnosed clinically

Against No history of arthritis, sore throats or other illness before onset of present symptoms of CHF 2 months before admission Only dubious evidence of active carditis (BSR variable, 7 to 34 mm per 1 hour Wintrobe, tachycardia, low irregular pyrexia, but bronchitis also present) *Clinically proved*

Case No 6 Fem, age 22 *For* CHF, regular rhythm, rough presystolic mitral murmur, accentuated P2 ECG, exaggerated and notched P waves Condition developed subsequent to first confinement 6 months ago

Against No history of previous illness other than occasional fever and body-aches *Doubtful, probable*

Notes on two cases not included in the statistical data for this investigation

Male, age 45, PM No 43/48 CHF with mitral and aortic stenosis, calcified mitral valve ring No Aschoff nodules or other histological evidence of active carditis (Fig 2)

Fem, age 40, PM No 9/49 CHF, mitral and aortic stenosis No Aschoff nodules or acute endocarditis

GROUP D FIJIANS CONGESTIVE HEART FAILURE DUE TO CHRONIC RHEUMATIC ENDOCARDITIS ?

Case No 1 Male, age 30 Auricular fibrillation, CHF (only systolic murmur heard) (Subsequently died in this condition—no PM permitted) *Doubtful, improbable*

Case No 2 Fem, age 26 CHF with regular rhythm, only systolic murmur heard No follow-up possible *Doubtful, improbable*

GROUP E INDIANS CHRONIC ENDOCARDITIS WITHOUT ACTIVITY OR DECOMPENSATION ? RHEUMATIC

Case No 1 Fem, age 24 Sought treatment for coryza and bronchitis Clinical, TRG and ECG evidence of mitral stenosis *Doubtful, probable*

GROUP F FIJIANS CHRONIC ENDOCARDITIS WITHOUT ACTIVITY OR DECOMPENSATION ? RHEUMATIC

Case No 1 Fem, age 30 *For* Cerebral embolism and hemiplegia History only of small pulmonary thromboses or possibly merely bronchitis prior to onset of CHF

murmur persisting over the 4 months of total observation period, becoming rougher towards end of the period. No pathogens in cultures of urine prostatic exudate stools, blood. Kahn negative Agglutination tests for typhoid and brucella negative.

Against Grating of both sternoclavicular joints. Gingivitis and foetor oris. TRG and ECG normal. No diastolic murmur *Doubtful probabl*

Case No 26. Fem., age 12. *For* Typical acute polyarthritis. Response to salicylates. History of similar illnesses 1 year and 2 years ago. Mitral systolic murmur constant, accentuated P2. TRG shows some enlargement to right and prominence in region of pulmonary conus. No pathogens in cultures of blood urine faeces. Typhoid and brucella agglutination negative.

Against No diastolic murmur ECG sinus tachycardia only No recurrences in 2½ months following discharge heart unchanged, TRG as before. *Clinically proved.*

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Case No 1 Fem, age 30 *For* Cerebral embolism and hemiplegia History only of small pulmonary thromboses or possibly merely bronchitis prior to onset of CHF

3 to 4 years ago, following birth of third and last child. PM (No. 85 48), mitral stenosis and calcification, admitting only tip of little finger tricuspid stenosis gross hypertrophy and calcification of left auricle (Fig 3)

Against: N history of arthritis or sore throats. No PM evidence of active carditis and no Aschoff nodules, recent or healed, in tissue from five optimum sites in the heart. *Doubtful probable*

Case No. 2. Male age 20 *For* Police recruit with typical clinical mitral stenosis confirmed by ECG

Against No history of rheumatic fever TRG shows only doubtful prominence of conus. *Doubtful, probable*

Note on case not included in the statistical data for this investigation Fem., age 40 PM No. 34/43 Death at home from abortion and haemorrhage Mitral stenosis. N CHF N Aschoff nodules in sections.

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TICK-BORNE RELAPSING FEVER IN SOMALILAND WITH SPECIAL REFERENCE TO THE BLOOD SEDIMENTATION RATE

BY

A D CHARTERS, M D (CAMB), M R C P , D T M & H ,*
Late Major, Medical Specialist

This paper has been compiled from investigations carried out in Somaliland during the years 1941 to 1945

Tick-borne relapsing fever in Somaliland differs from the louse-borne infection of the Abyssinian highlands in being far less severe and in having relapses of shorter duration There was not a single fatality out of the 42 cases treated , this series included four Europeans, 31 Somalis and seven East Africans Out of 140 pyrexial attacks, the average duration was 3 2 days, the course of an attack varying in individual cases from 1 to 17 days The interval between relapses also varied greatly, from 2 to 63 days, the average being 8 6 days The average number of attacks per patient was four, but one patient, a European, had as many as 13 bouts of pyrexia, and was in hospital for a total of 137 days (over 4 months) before he was fit for discharge

The disease was definitely more severe in the European cases than in the Somalis and East Africans One European developed the complication of iritis while another suffered from cerebral symptoms with intractable headache and vomiting, neck rigidity and positive Kernig's sign The latter patient, whose symptoms were relieved by lumbar puncture, showed a raised cerebrospinal pressure with an excess of cells and protein in his fluid

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A total of eight lumbar punctures was performed in this series, five of the patients suffering from neck rigidity and showing Kernig's sign. The C.S.F protein was increased in five of the cases (four of whom were suffering from neck rigidity) and there was a cobweb clot on standing in one case. Spirochaetes were not seen in films of the C.S.F but might have been demonstrated by animal inoculation.

Complications included the following: iritis in three cases, basal pneumonia in two, dysentery of the bacillary type (not responding to succinyl sulphathiazole) in two, diarrhoea in three others, acute nephritis in one, hepatitis with jaundice in one, herpes of the right auricle in one, left cervical neuritis in one, and unilateral otitis media in one.

An interesting case of Jacksonian epilepsy was seen. The patient was a Somali. It is unfortunate that, as no spirochaetes were recovered from his blood, the diagnosis could not be proved, but his relapsing type of pyrexia suggested that he was suffering from relapsing fever. The cerebrospinal fluid showed no abnormality and was not increased in pressure. Each fit commenced in the right hand and then spread rapidly up the arm and thence simultaneously to the right side of his face and lower extremity. The left side of the body was unaffected. There was no unconsciousness. The attacks rapidly succeeded one another and persisted with diminishing severity for 14 days. Between the spasms there was a flaccid upper motor neurone paralysis. After 7 weeks the fits, which had diminished in number under phenobarbitone, ceased altogether. The power came back in his right limbs, and within 4 days of his admission the abdominal reflexes had returned and the plantar responses had become flexor. He was discharged walking with a slight limp after 8 weeks in hospital.

The Wasserman reaction is said to be positive in about 20 per cent. of cases (MANSON BAHR, 1945; HEILMAN and HERRELL, 1943), but out of eight cases examined in my series the Kahn test was negative in all. The majority of these patients were natives of British Somaliland who, being strict Mohammedans, rarely suffered from syphilis. These findings are in agreement with those of GARNHAM *et al.* (1947) who during their investigations on a louse-borne epidemic in Kenya, found the Kahn reaction positive in only three out of 38 specimens of blood examined at different stages of the disease; they state that the positives were probably due to syphilis or yaws.

There is usually said to be a leucocytosis in this disease. Out of 26 leucocyte counts performed during the attack, the average total count was 7,795 (varying between 3,000 and 14,000) and the average neutrophil percentage 60 (varying between 45 per cent. and 90 per cent.). The neutrophil count varied from 1,740 to 10,920, the average being 4,399. Only three out of 18 cases had counts over 6,000 per c.mm. In this series, therefore, a neutrophilia was the exception rather than the rule.

There was commonly a secondary anaemia, the average red cell count being 4,140,000 per c.mm., the haemoglobin 71 per cent. (Sahli) and the colour index 0·84.

The blood sedimentation rate is so commonly employed nowadays as a sign of recovery from active disease, especially in rheumatic fever and tuberculosis, that I was tempted to investigate whether it might be of value as an

index of complete recovery or of the susceptibility to future relapses The technique employed in Somaliland was that of Westergren It was not possible to correct for anaemia, neither is such a correction entirely reliable, since the correction is based upon dilution of the blood which is not strictly comparable to clinical anaemia (DAVIS, 1946), and since no allowance is made for the natural power of anaemic blood to compensate for its tendency to rapid sedimentation (McFARLANE and O'BRIEN, 1946) Moreover, the Westergren is less susceptible than the Wintrobe method to the effects of anaemia (DAVIS, 1946)

One hundred and fifty-nine B S R examinations were performed, including 113 tests on Somalis, 27 on East Africans and 19 on Europeans The B S R taken during attacks varied greatly (from 4 to 110 mm.), the average of 19 examinations being 61 7 mm in 1 hour (49 2 in the Somalis, 78 in the East Africans, and 58 in the Europeans) (Tables I and II)

TABLE I AVERAGE B S R DURING ATTACKS

	Average B S R	Number of tests
European	58 0 mm	2
East African	78 0 „	5
Somali	49 2	12
Total	61 7 mm	19

TABLE II AVERAGE B S R. FOR EACH DAY OF ATTACK

Day of attack.	Average B S R	Number of tests
1st	79 8 mm	5
2nd	65 3 „	6
3rd	42 0	2
4th	4 0	1
5th	30 0	2
6th	110 0	1
11th	6 0 „	1

An attempt was made to ascertain whether the B S R dropped to normal between attacks or whether it could be used as an index of prognosis in the prediction of further relapses Examinations were therefore made at intervals for several weeks after the last bout of temperature The results are depicted in Table III

As shown in the table, there is a tendency for the figures to diminish after the 4th week, but there was still a tendency to a raised level even after 2 months There was, of course, great variation in individual figures A few cases dropped

permanently to normal after a few weeks, as far as could be calculated from estimations over a period of 3 to 4 weeks. An interesting point about these tests was the marked and sudden variations in B.S.R. without any *corresponding* rise of temperature or change in the patient's clinical condition. For instance one patient showed a rise from 22 to 90 mm. during the 3 weeks after his last attack, in spite of the fact that he was feeling perfectly well and was discharged to his unit after 4 weeks. Another dropped to nil in 3 weeks, rose to 80 in 4 weeks, and again dropped to nine in 5 weeks, without any *rise of temperature* or change in his general health. A third fell to one in 2 days, rose to 96 in 8 days, and again fell to four in 24 days. One patient had a B.S.R. of 112 mm. 10 days after his second attack and 6 days before his last rise of temperature to

TABLE III. [illegible] B.S.R. AFTER [illegible]

	East African.		European.		Somali.		Total.	
Weeks.	Average B.S.R. mm.	Number of tests.	Average B.S.R. mm.	Number of tests.	Average B.S.R. mm.	Number of tests.	Average B.S.R. mm.	Number of tests.
1st	40·9	10	34·7	9	[illegible]	[illegible]	37·4	51
2nd	33·7	6	[illegible]	3	32·0	—	[illegible]	37
3rd	51·0		13·5	2	13·7	18	28·1	24
4th	—	—	30·0	1	31·1	12	30·5	13
5th	—	—	—	—	11·0	7	11·0	7
6th	—	—	—	—	10·9	6	10·9	6
7th	—	—	—	—	17·3	3	17·3	3
8th	—	—	—	—	19	2	19·5	
9th	—	—	—	—	31·0	1	31·0	1

only 99° he felt very fit at the time of these tests. One had a very high rate for 3 weeks after his third attack rising to a maximum of 132 mm. 4 days before his last relapse in spite of the fact that he was feeling quite well in the intervals between his pyrexial bouts.

B.S.R. estimations were also made before relapses (Table IV) in order to find out whether there was any tendency for a rise to occur as the next relapse became more imminent. The average was 40·9 mm. 1 week, 46·5 mm. 2 weeks, and 41·5 mm. 3 weeks before the attack. There was, therefore, no tendency for the B.S.R. to rise as the time approached for the next relapse.

One is tempted, after studying the B.S.R. figures, which remain raised for weeks after all symptoms have subsided, to conclude that even after all clinical signs have disappeared, there still remains a latent infection in the body. Such a view is supported by the long interval which may sometimes occur between relapses (63 days in one case), and by the fact that spirochaetes have been isolated

from the brains of guineapigs 14 months after primary inoculation (MANSON-BAHR, 1945), and by the insidious manner in which the organism is transmitted by the tick through its ova even to the third generation, without the necessity for any intermediary host.

It appears that the spirochaete of relapsing fever resembles that of syphilis in its characteristic property of remaining latent in the body for long periods without causing symptoms. I had one patient who developed a bout of relapsing fever (confirmed by the discovery of spirochaetes in his blood) exactly 1 year after his previous attack; one wonders whether the second attack was a fresh infection or a very late relapse of his original illness. Another feature common to the spirochaetes of relapsing fever and syphilis is the neurotropic character

TABLE IV AVERAGE B S.R BEFORE RELAPSES.

Weeks before relapses	East African		European.		Somali		Total	
	Average B S R mm	Number of tests	Average B S R mm	Number of tests	Average B S R mm	Number of tests	Average B S R mm	Number of tests
1	50 5	9	47 0	4	25 3	18	40 9	31
2	62 7	3	55 0	2	21 7	8	46 5	13
3	88 0	1	19 5	2	17 0	1	41 5	4
4	—	—	—	—	—	—	—	—
5	—	—	—	—	20 5	2	20 5	2

and the tendency to latent infections in the nervous system. GARNHAM *et al* (1947), working in East Africa, found that neurotrophism was rare in the louse-borne spirochaete, although invariably present in the tick-borne variety. An interesting feature of the B S R figures is the curious way in which the B S R. fluctuates without any corresponding clinical signs of activity. This phenomenon suggests that fluctuation may occur in the intervals of the infection quite independently of the clinical features as indicated by relapses of pyrexia.

Treatment.—There have been so many reports about the value, or lack of value, of arsenical therapy that, at the suggestion of Brigadier E R CULLINAN, I decided to investigate its therapeutic action, employing untreated controls taken at random. These tests were performed at Mandera, in Somaliland, and at Diredawa, in Abyssinia, the patients including Europeans, East Africans and Somalis. Neosalvarsan gramme 0 6 was given intravenously at the onset of the first relapse, the primary attack being untreated on account of the difficulty of seeing the patient at the onset of his disease. Twenty patients were treated and 14 employed as untreated controls. The following were the statistics drawn

from 91 attacks of pyrexia amongst the treated cases and 49 amongst the untreated

Of the 20 treated cases, 18 relapsed after arsenical therapy

It must be concluded that at Mandera and Diredawa arsenical therapy cannot be claimed to have had any therapeutic value.

At Borama, in British Somaliland, on the other hand, and at Urso, in Abyssinia, four Free French Somalis were treated without a single subsequent relapse. Five patients were also treated at Yatta, in Kenya, four of whom responded without a recurrence and one had a single extremely mild relapse. QUIN and PERKINS (1946) working among African troops in East Africa, observed little benefit from N.A.B. injections, the 80 cases treated exhibiting the same average of two relapses as the 49 controls.

TABLE V THERAPEUTIC EFFECT OF INTRAVENOUS ARSENIC IN TICK-BORNE RELAPSING FEVER AT MANDERA AND DIREDAWA.

	Average number of attacks from onset.	Average duration of attack after first relapse.	Average maximum temperature after first relapse.	Average interval between attacks.	Average B.S.R. after 7 days from end of first relapse.
Treated cases	4·6	3·3 days	101·5°	10·3 days	56·7 mm.
Untreated cases	3·5	3·1	101·7°	7·3	48·7

It therefore appears that, in certain localities, arsenic did produce benefit, while in other districts it had no effect whatsoever The explanation probably lies in the fact that certain strains of tick borne spirochaetes have become arsenic-resistant, possibly through previous arsenical treatment of these strains, just as strains of gonococci became sulphonamide-resistant in Italy and elsewhere.

A similar theory probably explains the differing views with regard to the value of arsenic in the louse-borne disease for whereas the author (1942) found this drug to be of great value in the Soddu district of Abyssinia, and GARNHAM, DAVIS, HEISCH and TIMMS (1947) found it of benefit in the Kenya disease WOLMAN (1944) claimed that it had no effect in Addis Ababa.

Penicillin was administered to two patients with tick borne relapsing fever both Europeans, in the dosage of 15,000 units every 3 hours for 6 days. One of the patients, whose treatment commenced during the sixth attack, developed seven subsequent relapses, while the other treated during his fifth attack, suffered no further pyrexia.

The general impression was that penicillin, in the dose given, was ineffective in preventing relapses. This would be expected in view of the results of animal

(2) The Kahn test was negative in the eight cases examined.

(3) In this series, neutrophilia was the exception rather than the rule

(4) B.S R. estimations were made during, and for several weeks after attacks. There was a tendency to a raised rate for several weeks after an attack, and to fluctuation in the B.S R. without any corresponding clinical signs of activity

(5) It is believed that a latent infection persists in the body (possibly the brain) for a considerable period after all clinical signs have disappeared. An analogy is drawn to the latency and neurotropic character of *Treponema pallidum*

(6) Arsenical therapy was of benefit in some areas, while proving of no value whatsoever in other districts, the explanation probably being the occurrence of arsenic resistant strains of spirochaetes in certain localities.

(7) Penicillin did not prevent relapses.

REFERENCES

CHARTERS, A. D (1942) *Trans. R. Soc trop Med Hyg* 35 27

DAVIES, L. J (1946). *Practitioner* 157 15 and 16

EAGLE, H., MAGNUSON H. G & MUSSELMAN A. D (1944). *U.S Pbl. Hlth Rep* 59 No. 18.

GARNHAM P C. C., DAVIES, C. W HEISCH, R. B & TIMMS G L. (1947). *Trans. R. Soc trop Med. Hyg* 41 151

HEILMAN, R. & HERRELL, W E. (1943) *Proc. Mayo Clin* 18, 457

INGRAHAM, H. S & LAPENTA, R. G (1946). *U.S Nav Bull* 46 1719 (quoted by *Trop Dis. Bull.* (1947). 44 17).

LEVADITI C. & VAISMAN, A. (1947). *C.R. Acad Sci* 225, 769 (quoted by *Trop Dis. Bull* (1948). 45 182).

LING, C. C. (1947) *Chin. med J* 65, 225 (quoted by *Trop. Dis. Bull.* (1948). 45, 343).

MANSON BAHR, P H (1945). *Manson Tropical Diseases* 12th Ed. pp. 188 and 196 London Cassell & Co.

McFARLANE, R. G & O'BRIEN, J R. (1946). *Practitioner* 157 1.

MURRAY C. (1947) *Clin. Proc Cape Town*, 6 113

MUWAZI, E. M K. (1946). *East Afr med J* 23 55

POSPELOVA-STROM H. S & TIBURSKAYA, N A. (1946) *Med. Parasitol Moscow*. 15 54 (quoted by *Trop Dis. Bull* (1947) 44 213).

QUIN E. C. & PERKINS, E. S. (1946). *J trop Med Hyg* 49 30

WOLMAN M. (1944). *East Afr med J* 21 1

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PALUDRINE TREATMENT OF EXPERIMENTAL MALARIA INFECTION, EFFECTIVE MINIMUM DOSES

BY

M CIUCA,
L BALLIF
AND
M CHELARESCU,
WITH THE CO-OPERATION OF
A TIMISESCU,
F VASILIU-MUNTEANU,
AND
M VRABIE TROFIM,
Malaria Study Centre " Socola " and I Cantacuzino Institute, Rumania

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The general conclusions given by Fairley (1946)* confirm the schizonticide action of paludrine in achieving, in some infections, even radical cure This drug is now one of the most efficacious for the treatment of malarial infection, since it has no toxic properties and is of high therapeutic value

Its efficacy, however, is related to the duration of the treatment, and we must not forget that patients are not inclined to submit to a long course of treatment once the temperature has reverted to normal On the other hand, the cost of the drug, to which must be added the general costs involved in its application, should also be taken into account in connection with mass treatment We publish the results of our work on cases of experimental infection with *Plasmodium vivax* and *P falciparum* in the hope of contributing something to the technique of this treatment

(*I*) *P vivax* Infection

Patients whose mental condition required malariotherapy were infected by inoculation with blood containing our old " BT$^{\mathrm{BOHR}}$ " strain, which originated in Madagascar

* *Trans R Soc trop Med Hyg*, **40**, 105

Paludrine treatment was administered at various stages of the infection in accordance with the duration required for the attack in terms of malariotherapy and the patient's general condition. Doses varying from mg 100 to 300 daily were administered, the treatment lasting from 7 to 15 days.

Table I gives *results* of the treatment in terms of forms of infection, duration of fever (in days) at the beginning of the treatment, and the dose employed.

TABLE I. (*P. vivax.*)

Patients.	Method of infection (by inoculation of infected blood).	Incubation in days		Form of infection.	Treatment paludrine.		Time-lag before disappearance in days.	
		Parasite.	Fever		Dose mg. × days.	Begun day of fever	Fever	Parasites.
1 C.C.	I.	3	4	A+P+	100 × 7	7th	1	5
2 C.R.	I.m.	7	10	A+P+	200 × 7	7th	2	6*
3 M.D.		10	13	A+P+	200 × 7	8th	1	6
4 D.V.		11	10	A+P+	200 × 7	9th	1	6
5 F.G.		4	5	A+P+	200 × 7	11th	1	7
6 T.O.	I.	1	1	A+P+	200 × 7	8th	2	7
7 S.E.	I.m.	10	10	A+P+	200 × 7	10th	1	4
8 D.M.	I.	9	12	A+P+	200 × 7	10th	1	5
9 C.P.		3	2	A+P+	200 × 7	10th	1	6
10 L.L.	I.m.	10	10	A+P+	200 × 7	10th	1	5
11 F.C.	I.		2	A+P+	300 × 15	7th	1	9
12 L.M.		9	9	A+P+	300 × 15	10th	1	8

* Parasitic relapse following inoculation with *P. falciparum*.
Abbreviations: I. = Intravenous. I.m. = Intramuscular.

Summary.—Twelve subjects were treated by malariotherapy, the infection being induced by inoculation of infected blood (*P. vivax*) intramuscularly or intravenously. All developed the disease (A + P +) after an incubation period of varying length, according to the method of inoculation.

Paludrine treatment was administered from the 6th to 11th day of fever onwards. The daily doses of paludrine given varied as follows: mg 100 (one patient), mg 200 (nine patients), mg 300 (two patients). The duration of the treatment varied from 7 days (ten patients) to 15 days.

In ten patients the fever disappeared generally 24 hours after the administration of the first dose of the drug; two of the 12 patients treated had no fever after the first two doses of paludrine. The disappearance of parasites from the blood was observed in a thick drop after intervals varying from 4 to 9 days. The average was 6·4 days from the administration of the first dose of the drug. A parasite count also showed the presence of degenerate parasites and shadows.

In infections with virulent blood, quantities of paludrine varying from mg 100 to 300 daily, and administered for a period of 7 to 15 days, have shown similar therapeutic efficacy

(*II*) *P falciparum* Infection

Twelve patients, infected with our strain MT[78] in order to induce therapeutic malaria, were treated with daily doses of mg 200 to 300 of paludrine for a period of 5 to 10 days Five of these were infected with blood and seven with sporozoites

Table II gives details of the development of the infection, and the results of the treatment

TABLE II (*P falciparum*)

Patients	Manner of infection	Duration of incubation in days		Form of infection	Treatment-paludrine		Time-lag before disappearance in days		
		Parasites	Fever		Dose mg × days	Begun day of fever	Fever	Trophozoites	Gametocytes
1 A D	B, i v	2	5	A+P+	100×7	15th	1	7	53
2 V M	B, i m	16	19	A+P+	200×7	5th	2	4	28
3 A V	"	13	15	A+P+	100×7	9th	1	3	36
4 C I	"	9	8	A+P+	200×7	8th	2	5	30
5 R.M	"	16	18	A+P+	200×7	6th	2	5	47
6 O S	200,000 Sp, i v	25	29	A+P+	100×7	11th	1	4	31
7 L N	300,000 Sp, i v	16	16	A+P+	200×7	7th	1	4	44
8 C M	Rep sp, i v	15	15	A+P+	300×5	7th	3	5	*
9 P D	,	30	35	A+P+	300×10	4th	2	8	*
10 P G	"	17	20	A+P+	300×7	5th	1	7	*
11 N M	"	13	13	A+P+	300×10	15th	1	9	*
12 P V	"	13	14	A+P+	300×10	7th	2	8	48

* Gametocytes present when patient was discharged after treatment.

Abbreviations B Infected blood; I m Intramuscular; Sp Sporozoites; Rep Repeated inoculations; I v Intravenous

Briefly, the average time required for the disappearance of fever and trophozoites in the five patients inoculated with virulent blood and treated for 7 days with mg 100 to 200 of paludrine daily, was 1 6 day for the disappearance of fever, and 4 8 days for that of the trophozoites All five patients showed the presence of gametocytes for a long period

In the seven patients inoculated with sporozoites, the average times required were 1 5 day for the fever to abate, and 6·4 days for the disappearance of the trophozoites.

All were gametocyte carriers. These patients had been previously given prophylactic treatment with paludrine which was not effective and did not affect the experiment.

A dose varying between mg 100 and 300 of paludrine daily and a treat ment lasting between 5 and 10 days, did not give rise to any differences in results.

Conclusions.

These experiments confirm the effective schizonticide action of paludrine. The number of cases treated is too small to permit of a comparison with other schizonticide substances. This treatment does not prevent the development of gametocytes.

the vessel. Oedema, often to an extensive degree, is an essential feature of the condition. For instance, swelling of the thigh and leg occurs with femoral thrombosis and of the arm, forearm and hand with subclavian thrombosis. In such a thrombophlebitis, an arterial spasm or arterial arteritis may be seen (MANSON BAHR and CHARTERS, 1946 and GELFAND, 1948 1949) and occasionally gangrene may follow (MANSON BAHR and CHARTERS, 1946 and GELFAND 1947) It is not difficult, therefore to appreciate that should the blood supply to a muscle be interrupted a liquefactive necrosis might ensue Such a condition would be described as a variety of so-called tropical myositis.

MANSON BAHR and CHARTERS (1946) FISHER (1941) and FISHER and LENDRUM (1947) state that suppuration does not occur in tropical phlebitis. They admit, however that its distinction from tropical myositis is sometimes difficult. FISHER (1941) noted the difficulty of differentiating between phlebitis of the common jugular vein and tropical myositis of the sternomastoid muscle. MANSON BAHR and CHARTERS find the oedema more extensive in tropical phlebitis than in tropical myositis. In tropical myositis peripheral oedema is absent.

The case reported in this paper was clinically one in which a femoral thrombosis of the right lower extremity took place. The whole of the limb was oedematous and the degree of swelling clearly not that normally associated with tropical myositis (Fig). After several days, softening with fluctuation appeared in the posterior aspect of the thigh. On aspiration, brownish-red pus was obtained—typical of that so often seen in tropical myositis.

It was clear that the abscess had to be drained. I felt that it would be of interest to the surgeon, during operation, to determine the condition of the femoral vein. This was performed by Dr GIBSON a Government Medical Officer who opened into Hunter's canal in the thigh and found a thrombosed femoral vein, which had in part become necrotic. The muscles had softened and appeared gangrenous.

CASE REPORT

The patient was a young male African aged about 25 years. H was admitted to the Salisbury Native Hospital on 1 4 49 with the history of swollen right leg of 4 days duration. H had first experienced pain in the groin and then noticed that the whole of his thigh and leg had become swollen. Walking was difficult on account of pain and swelling Otherwise he felt well.

On examination the patient, who was well covered, looked fit with no obvious sign of nutritional disease. His temperature on admission was 101 F increasing with daily excursions to 103° F and 104 F and continuing for about weeks after which it gradually subsided to normal. The tongue was fairly clean.

There was an extensive oedematous swelling of the lower right limb, particularly of the thigh, and marked tenderness of the right leg, especially along the saphenous vein (Fig.). The foot in direct line with the thigh and leg was slightly swollen as well. The measurements of the two limbs were as follows :—

	2 in. above external malleolus.	*1 in. below tibial tuberosity*	*3 in. above patella.*
Right lower extremity	8½ in.	14 in.	17½ in.
Left	8	12	15½ ,,

The results of investigations performed during his stay in hospital were

(1) *Blood smears* —Negative for malaria and relapsing fever parasites
(2) *Two blood cultures* (taken on admission) —Both negative
(3) *Agglutination tests* (Widal and Weil-Felix) —Negative
(4) *Blood Wassermann reaction* —Negative
(5) *Urine* —Clear of sugar and albumin Microscopical examination of urine negative
(6) *Stool* —Ova of *S mansoni* and hookworm were identified
(7) *Radiograph of chest* —Normal

FIG —Showing the extent of the oedema in the right lower extremity

On 6.4.49 we observed that the thigh was still swollen, especially in its posterior aspect which was tender and fluctuated. As an abscess was suspected, the site was aspirated, releasing pus of brownish-red colour and of thick consistency. A film was taken and stained, revealing fairly numerous polymorphonuclear leucocytes and few mononuclear cells. On culture pure growth of *Staphylococcus aureus* was obtained.

It was decided to determine the condition of the femoral vessels, in case these were thrombosed. On 11.4.49 Dr. R. S. Gibson performed this exploratory operation of the thigh, selecting Hunter canal. Here he found the muscles to be necrotic with much pus in the area. The femoral artery pulsated, but the vein felt thrombosed and was necrotic in part. The abscess in the thigh was drained. The patient made an uneventful recovery and was discharged from hospital on 29.4.49.

Two small portions from the necrotic region of the vein were taken at the time of operation for section. Unfortunately the one with the wall itself was accidentally mislaid and the other contained only the thrombus and the surrounding tissue. A haematoxylin and eosin slide of this was sent to Professor A. C. Lendrum, who kindly reported on it as follows:

"... in the adjacent fatty areolar tissue there is an actively cellular reaction increasing in intensity towards two edges of the fragment of tissue where there is mass of blood and clot of fairly recent date. The section does not allow one to say that this clot is lying within any definite structure although it is possible that the wall of vein may have lain where there is now this cellular inflammatory zone. The main feature of this is the predominance of large vesicular nuclei of endothelial type some of which are lining capillary blood vessels. No larger or aberrant forms are seen and with this method of staining it is of course not possible to pick up inclusion bodies, but in general the appearances are not incompatible with those that the Drs. Fisher and I described."

Comments.

There would appear to be suggestive evidence that at least one of the varieties of tropical myositis belongs to the group of conditions included in the term "primary tropical phlebitis or angitis." Clinically in view of the very extensive oedema of the lower extremity the case recorded in this paper appeared to be one of a femoral thrombosis. The subsequent abscess formation in the posterior aspect of the thigh pointed to some interference with the blood supply. This was borne out by the finding of a thrombosed vein in Hunter's canal.

It should be noted that this article is not intended to postulate that all cases of so-called pyomyositis tropica originate in this manner but merely to suggest that some may. It is quite probable that a number of different aetiological factors are responsible for the condition.

It is hoped that others interested in this field of work will investigate the problem further and perhaps corroborate or disprove the suggestion made. This line of research might be usefully carried out both in sub-tropical Africa and elsewhere.

References.

Fisher, A. C. (1941). *S. Afr. med. J.* 15, 131.
——— Fisher, M. M. & Lendrum, A. C. (1947). *J. Path. Bact.*, 59, 405.
Gelfand, M. (1947). *Trans. R. Soc. trop. Med. Hyg.* 40, 789.
——— (1947). *Lancet* 2, 804.
——— (1947). *Brit. med. J.* 2, 847.
——— (1949). *S. Afr. med. J.* 23, 129.
Manson-Bahr, P. E. C. & Charters, A. D. (1946). *Lancet* 2, 333.

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CAPILLARIA HEPATICA (BANCROFT, 1893)

A CASE REPORT

BY

GUSTAV ENGLER, M D , F I C S ,

AND

GUILLERMO SANCHEZ

(From the Almirante Hospital of the United Fruit Company Panama Division)

Ever since MACARTHUR (1924) described an authentic case of fatal human infection with *Capillaria hepatica* (Bancroft, 1893) in a British soldier in India, the interest in this nematode has been active The subsequent cases which number now over 30, were probably spurious infections caused by ingestion of the liver of certain infected animals The parasites seem to be ubiquitous throughout the tropics

FOSTER and JOHNSON (1939) investigated the probable source of infections in Panama and traced their cases to infection from the liver of the white-lipped peccary (*Tayassus pecari spiridens*) of the red spider monkey (*Ateles geaffroyi*) and of the capucine monkey (*Cebus capucinus imitator*) BROSIUS (1948) added to the possible list of sources of infection, the wild turkey (*Meleagris gallopavo silvestris*) the " tipesquinte " (Order Marsupalia) and the " paujui " a species of pheasant

During the investigation of the present case all members of the patient's household were examined but no other case of *Capillaria hepatica* infection could be found

CASE REPORT No 14143

Alejandrina S , 19, Panamanian, female, housewife, resident of Almirante, was admitted on 2nd March, 1949

Family history patient's household includes the persons listed below, all provided for by patient's father, a working man and occasional hunter in the vicinity of Almirante The animals brought home during the last 12 months were wild hog, tipesquinty, deer, wild turkey and mountain hen The names are given as described by the patient The liver of these animals was fried in sesam oil in an iron pan on charcoal fire, and was allegedly always well done and never raw

Personal history.—Kahn 4 plus 1947; full course of antiluetic treatment; now negative. Normal confinement of first child November 1945.

Present history.—Fever cough for 2 days, sputum streaked with blood.

Examination.—110 lb; blood pressure 112/70 temperature 100° Fairly nourished young woman, scar after burn of upper and lower lip pharynx injected; chest, abdomen, pelvic organs without abnormal findings. Laboratory findings: Urine normal red and white blood count within normal limits Kahn test negative, blood smear for malaria negative Stool: *Necator americanus* *Trichuris trichiura* and *Capillaria hepatica* (ova) Chest film normal.

Course.—A total of six stool specimens were examined before anthelmintic treatment was instituted. All were positive for *Capillaria hepatica*. Through the courtesy of Dr H CLARK the Gorgas Memorial Laboratory examined a specimen and confirmed the diagnosis.

The upper respiratory infection cleared under penicillin and sulphonamides. Following Brosius suggestion chenopodium treatment was administered (chenopodium gtt. xxx, followed by castor oil oz. ii after 2 hours) but this treatment failed to eliminate the ova in our case. One week after the chenopodium treatment the stool specimen still showed numerous ova of *Capillaria hepatica*

Hexaresorcinol gramme 1·0 (Cystoid Sharp and Dohme 5 × 0·2) followed by cathartic compound after 12 hours, cleared the infection and the stool specimens have remained negative since then.

A stool survey of other members of the household was then undertaken —

Emilio M., father 39	No parasites found.
Augustina, mother 45	*Ascaris lumbricoides*.
Alfonso M., 8	*A lumbricoides*; *Trichuris trichiura*.
Fortunato M., 7	" *T trichiura*.
Inez M. 11	*T trichiura*.

SUMMARY

Another case of probably spurious infection with *Capillaria hepatica* (Bancroft) is presented in the members of a household, where certain wild animals are consumed. The other members of the household were found free from *Capillaria hepatica*. Chenopodium in average doses failed to eliminate the infection. Hexaresorcinol in standard doses eliminated the ova of *Capillaria hepatica* from the stools.

REFERENCES

BELDING, D L. (1942). *Text Book of Clinical Parasitology* 287 New York & London D Appleton-Century Co Inc.

BROSIUS, O T THOMAS, E. & BROSIUS, B. (1948). *Trans. R. Soc. trop. Med. Hyg* 42 95

CRAIG C. F & FAUST E. C. (1943). *Clinical Parasitology* 3rd Ed., 243. London Henry Kimpton.

FAUST E. C. & MARTINEZ, W H (1935) *J Parasit.*, 21 232.

FOSTER, A. O & JOHNSON, C. M. (1939). *Trans R. Soc. trop Med. Hyg* 32, 639

MACARTHUR, W P (1924). *Proc R. Soc. Med.*, 17 83.

STRONG, R. P (1945). *Stitt's Diagnosis Prevention and Treatment of Tropical Diseases*, 7th Ed., 1298. London H. K. Lewis & Co.

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ANNOUNCEMENTS.

NEXT MEETING OF THE SOCIETY

The next meeting will be held at Manson House, 26, Portland Place, on Thursday, 16th February, 1950 Dr JOSEPH E SMADEL, Chief of the Department of Virus and Rickettsial Diseases, Army Medical Centre, Washington, D C, will read a paper entitled "Chloramphenicol (Chloromycetin) and Tropical Medicine"

MANSON LECTURE

To perpetuate the memory of the late Sir PATRICK MANSON, the Council of the Society has decided to establish a MANSON LECTURE FUND, to which subscriptions are now invited It is hoped to raise a sum of at least £2,500, the accumulated interest from which will be devoted to financing a Manson Lecture

The Lecture will deal with some aspect of tropical medicine or hygiene and will be given periodically by a recognized authority The lecturer and the subject on which he will be invited to speak, will be decided by the Council of the Society

The Manson Lecture will be open to all members of the medical profession and will be advertised in the general medical press, in which it may be subsequently published

MOVEMENTS OF FELLOWS

The following Fellows from abroad have notified the Secretaries that they are *temporarily* in the British Isles Letters addressed to any of these care of the Royal Society of Tropical Medicine and Hygiene, Manson House, 26, Portland Place, London, W 1, can usually be forwarded to the home address

To ensure the accuracy of this list, Fellows named below are particularly requested to advise the Secretaries when they return to their stations abroad

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BARNES, G T, Fiji
BLOMFIELD, D M, Kenya
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BRAINE, G I H, Malaya
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BUNNY, R S, Kenya
CALWELL, H G, Tanganyika
CAMPBELL, G, Trinidad
CHANG, HOEY CHAN, Malaya
COOPER, P R, Nigeria
DAVIDSON, Lt-Col T J, India
DICKIE, ROBERT, Nigeria
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ELMES, B G T, Canada
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WILSON, D BAGSTER, Tanganyika
WISEMAN, R H, Kenya
WORTH, H N, South Africa
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NEW FELLOWS

At the meeting of the Society held at Manson House on 19th January 1950 the following 23 candidates were elected Fellows of the Society —

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BONP GEORGES J M.D D.SC., Professor Inst. Med. Trop Antwerp
BRADLEY ROBERT M.B., CH.B. (N.Z.) England.
CULLINAN EDWARD R., M.D (LOND) F.R.C.P (LOND.), England.
EVENS, FRANS M. J C., M.D (GHENT) Trop D (Antwerp) Belgium.
GAMONDI, PIETRO, M.D. D.T.M (ROME), D.T.M (LISBON), Italy
GROCOTT ROBERT G Med. Technologist B.SC. (Biology) Panama.
JORDAN, PETER, M.B., B.S. (LOND), M.R.C.S. (ENG) L.R.C.P (LOND) England
KABIR, AHMED M.B., B.S. (P TNA) Pakistan.
MARSHALL, J T M.B., B.S. (MADRAS), Surg Lt. R.I.N., India.
MEIRA, J A., M.D (SAO P ULO), Professor f Diagnostic and Infectious Diseases, Univ Sao Paulo, Brazil.
MIRLOW JOHN M.D. (TOULOUSE), England.
MURPHY ROBERT R., M.D., California.
PANDIAN, A. J YA, M.B., B.S. (MADRAS) Ceylon
PRASAD HARI, M.B. B.S. (P TNA) D.T.M. (CAL.), Bihar India.
RAGAB HUSSEIN A. A., M.B., CH.B. (CAIRO), D.T M. & H. (ENG.) PH.D. (LOND) Egypt.
RONSSE, CALLIATE, M.D. Belgian Congo.
SAVAGE, RICHARD G A., F.R.C.S.E., M.B CH.B. (EDIN.) Nigeria.
SCHNEIDER, JEAN M.D Chef de Clinique Fac. Med., Paris.
TURNER, LESLIE H., M.R.E., M D. (LOND.) D T.M. & H., Malaya.
VAN YENDYCK, GUY M.D. Belgian Congo.
YATES, J VES, M.B., B.S. (MADRAS) Ceylon.
YOUNG NORMAN A. F M.B., B. (LOND.) M C.S. (ENG) L.R.C.P (LOND.) Persian Gulf

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All registered medical and veterinary practitioners and others interested in scientific pursuits relating to tropical medicine, whose qualifications are deemed satisfactory by the Council, are eligible for election as Fellows of the Society

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THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

ZO'AE TORRIDAE TUTAMEN

PATRON HIS MAJESTY THE KING

Number on Register of Fellows

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Name in Full (In Block Capitals)	Titles, Qualifications, etc, and their Source	Home Address *	Address Abroad *

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1 ________________ *Signature of Fellow* | 2 ________________ *Signature of Fellow*

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Papers submitted for publication in these TRANSACTIONS should be sent to the **Secretaries, Manson House 26, Portland Place London, W.1**

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The abbreviations used are those shown in the **World List of Scientific Periodicals**, 1934, w conforms to the rules of the *International Code of Abbreviations for Titles of Periodicals*, 1930. In general nouns have capital, adjectives small, initial letters; articles, conjunc and prepositions are omitted; the place of imprint is added only when uncertainty might arise,

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Bull Soc Path. exot	*Indian med. Gaz*	*Z. Hyg InfektKr*

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centigramme, cg.	kilometre km.	millimetre mm
centimetre cm.	micron μ.	ounce, oz.
cubic centimetre, c.c.	microgramme, μg.	pound lb
cubic millimetre, c.mm.	milligramme mg.	
kilogramme kg	millilitre ml.	

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NOTICE.—The attention of those who propose to contribute papers for publication is directed to "Editorial Notices" on page xx

TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

MARCH, 1950 VOLUME 43 No 5

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[*The previous number of these Transactions, Vol 43, No 4 was published on 28th January, 1950*]

TRANSACTIONS
OF THE
ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

VOL 43 NO 5 MARCH, 1950

ORDINARY MEETING

of the Society held at

Manson House, 26, Portland Place, London, W ,

on

Thursday, 19th January, 1950, at 7 30 p m

THE PRESIDENT,

Professor H E SHORTT, C I E , M D , D SC , D T M & H , Colonel I M S (retd), in the Chair

PAPER

THE MALARIA EPIDEMIC OF 1943-1946 IN THE PROVINCE OF NORTH-HOLLAND

BY

N H SWELLENGREBEL
Institute for Tropical Hygiene, Amsterdam

Before I read my paper I think it will be appropriate if I say a few words about one of the Honorary Fellows of this Society, who died on the 24th of December last year I speak of Professor WILHELM SHÜFFNER In Holland we honour him as a man who was amongst the first, if not the first (1897–1902), to prove on a large scale that the so-called tropical diseases can be prevented without any change in the "murderous" climate He was nearly eighty-three when he died after a short illness, and it is greatly to be regretted that the last years of his long life were darkened and saddened by events outside his control I wish to testify to my sincere friendship and great admiration for the scientist who has left us Thank you, Mr President, for allowing me to say these few words

Eleven years ago MARTINI censured DE BUCK and me for the title of our book, "Malaria in the Netherlands." It was all about North-Holland, he said. His blame was unfounded, as we had given the other provinces their due. In the present instance I am avoiding any disapproval of that kind by the title I chose for my paper.

Malaria in North-Holland is transmitted by *Anopheles maculipennis* and is mainly caused by *Plasmodium vivax*. Quartan is extremely rare, falciparum malaria is not indigenous. Mortality due to indigenous malaria is practically non-existent.

All this applies to the present century only. I am not concerned with the history of malaria.

(1) PERIODICITY THROUGH SUCCEEDING YEARS

There is one place only in North-Holland where malaria has been kept under observation from 1902 onward. That place is the village of Wormerveer to the north of Amsterdam. There we know for certain that there have been three major epidemics of malaria. The first had already passed its maximum when the observations commenced, the second reached its peak in 1922, and the third in 1946. So there were peaks in 1901, 192[illegible] and 1946 at intervals of 21 and 24 years (Table I and Graph 1).

TABLE I.

[illegible]NUAL NUMBER OF MALARIA PATIENTS IN THE VILLAGE OF WORMERVEER†

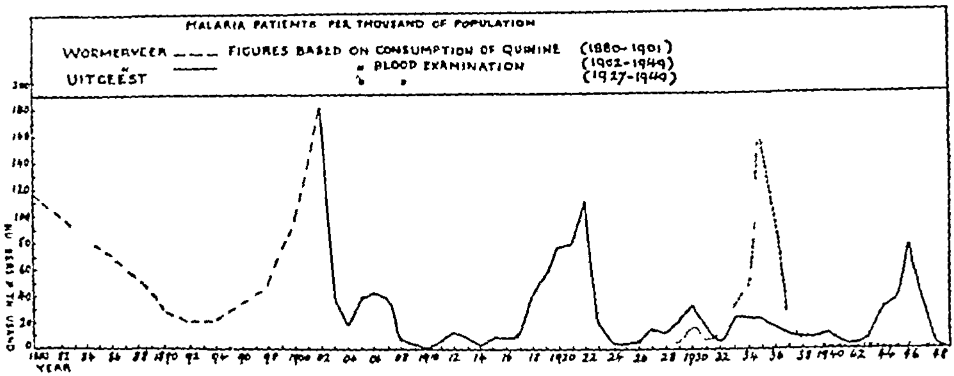
Year	Number of patients.	Year	Number of patients.	Year	Number of patients.
1902	540*	1918	117	1934	157
1903	111	1919	173	1935	161
1904	86	1920	220	1936	115
1905	119	1921	234	1937	77
1906	126	1922	326*	1938	64
1907	111	1923	64	1939	[illegible]
1908	25	1924	12	1940	[illegible]
1909	[illegible]	1925	13	1941	1
1910	3	1926	10	1942	4
1911	10	1927	65	1943	54
1912	40	1928	68	1944	75
1913	[illegible]7	1929	110	1945	334
1914	10	1930	201	1946	494*
1915	[illegible]8	1931	104	1947	303
1916	23	1932	[illegible]3	1948	1
1917	[illegible]7	1933	164	1949	

* Major epidemic.

† Till 1926 the figures apply to a population of round about 3 000. From 19[illegible] onward they apply to a population of 6 000 gradually rising to 10 000.

KORTEWEG, to whom we owe the observations up till 1918, had been practising medicine in Wormerveer long before 1902 So he was cognizant of an earlier major epidemic, which occurred in 1880 However, he refused to accept the diagnosis of malaria as valid, unless supported by the finding of parasites Thus he rejected all his observations previous to 1902 Otherwise we might have added 1880 to the other peaks, and another interval of 21 years between 1880 and 1901

GRAPH I



The figures in Table I are absolute figures Those of 1902 and 1922 refer to a population of 3,000, those of 1946 to 10,000 inhabitants, so the incidence during the peak years was 18 per cent, 11 per cent, and 8 per cent

Although there are no continuous observations to match those collected in Wormerveer, we know that cases of malaria were uncommonly numerous in many other villages and towns of North-Holland in 1900 to 1902, in 1919 to 1922 and in 1943 to 1948 It is only since 1927 that fairly exact figures are known for a number of villages besides Wormerveer The city of Amsterdam has records from 1920 onward (Table II and Graph II)

The Netherlands are a very small country, and North-Holland is extremely small, so one might have expected that malaria conditions are fairly homogeneous throughout the whole province, but they are not Not only have some villages much malaria and others little, but in the villages with much malaria the peak years are not always the same The malaria wave in some villages does not always rise and fall synchronously with that of the others As an instance I may mention the village of Uitgeest, less than 4 miles north of Wormerveer It had its own private peak year in 1935, with a malaria incidence of 16 per cent of the population

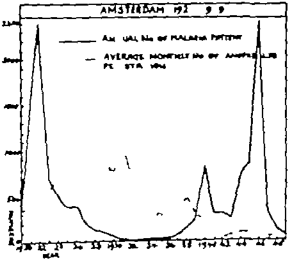
This lack of synchronism in the rise and decline of major malaria epidemics, even in neighbouring villages, is a serious inconvenience if one wishes to assess the effect of control measures Take that same village of Uitgeest Control

TABLE II.

ANNUAL NUMBER OF MALARIA PATIENTS IN THE CITY OF AMSTERDAM ([illegible] INHABITANTS). AVERAGE MONTHLY NUMBER OF ANOPHELES IN EACH CATCHING STATION.

Year	Number of patients.	Average monthly number of anopheles.	Year	Number of patients.	Average monthly number of anopheles.
1922	2,391	296†	1936	51	222
1923	472	370	1937	69	273
1924	535	1 018	1938	156	490
1925	412	820	1939	245	307
1926	403	483	1940	625	230
1927	225	585	1941	300	198
1928	142	815	1942	304	54
1929	118	930	1943	251	73
1930	73	773	1944	776	93
1931	41	1 031	1945	963	91
1932	15	782	1946	[illegible],455	267
1933	41	242	1947	370	49
1934	45	234	1948	133	90
1935	43	328	1949	51	—

Major epidemic, in 1920 and 1921 247 and 1 680 respectively
† In 1920 and 1921 320 and 428 respectively



GRAPH II

had dropped to 2 per cent, in 1939 to 0 5 per cent From 1940 until 1949 it has never risen above 0 3 per cent Most of the villages in the neighbourhood were having their peak year Still, how are we to know that it will not have another peak year of its own round about 1955? We cannot be sure till then That is the reason I shall feel very diffident when I come to tell you about our control measures and their results But I have not yet arrived at that stage

Whether synchronous or not, there is no doubt that there exists a periodicity in the occurrence of major epidemics, and it is not over bold to assume that the periods have a length of 20 to 25 years The last two major epidemics coincided with the end of two world wars, but the first one (I discard the 1880 epidemic) did not The last two also coincided more or less with considerable malaria epidemics in other European countries I do not know that the first one did In any case, the explanation offered in other European countries for the existence of these epidemics does not apply to North-Holland, as far as the first world war is concerned After that war our system of drainage of the land had not been damaged There were no malaria infected soldiers returning from the war

After the second world war explanations abounded The Germans had flooded part of the country in 1944, and it was not till the end of 1945 that the land was dry again, but, as far as we could judge, this damage done to the land had not raised the numbers of anopheles (Tables and Graphs II and III) As a matter of fact, flooding had destroyed the breeding places, which do not occur in large stretches of shallow water soiled by decaying vegetation, but

TABLE III

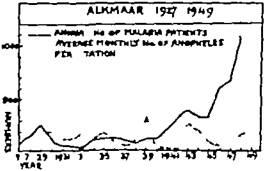
ANNUAL NUMBER OF MALARIA PATIENTS IN THE TOWN OF ALKMAAR (39,240 INHABITANTS) AVERAGE MONTHLY NUMBER OF ANOPHELES IN EACH CATCHING STATION

Year	Number of patients	Average monthly number of anopheles	Year	Number of patients	Average monthly number of anopheles
1927	81	—	1939	120	342
1928	151	—	1940	126	44
1929	245	178	1941	224	17
1930	102	188	1942	313	26
1931	63	123	1943	391	262
1932	54	134	1944	310	201
1933	55	181	1945	331	62
1934	108	124	1946	604	58
1935	126	178	1947	650	105
1936	127	106	1948	1,084	142
1937	93	54	1949	65	173
1938	84	115			

in the water of the drainage ditches, kept clean and aerated by living aquatic vegetation. Moreover in some places the epidemic started well before the flooding (Tables II and III)

Another apparent reason for the 1948 epidemic was the fact of people returning from the Japanese concentration camps in Indonesia, Burma and Siam. From January 1946 onwards hundreds came to this country many of them carrying plasmodia (almost invariably vivax). But that happened at a time when the epidemic had almost reached its peak.

Finally whatever one's opinion may be on the possible effect of these war and post war conditions, one has always to face the fact that there was no war in the years between 1900 and 1905 nor any other serious disturbance to explain the major epidemic of 1900 to 1902.



Graph III

My opinion is that the epidemics of 1919 to 1922 and of 1943 to 1948 would have happened just as well without any war. Their cause is not to be looked for among extraordinary conditions, but among always recurring conditions, having their place in the ordinary course of events.

What are these conditions? The first thing that comes to the mind is the density of the anopheline fauna. We have gone into that in the accustomed way by regularly examining catching stations. That is a comparatively easy matter if the malaria periods are of the usual length of 5 years or less, but to keep a catching station going for 20 years is quite another proposition. We lost them, took another lost that again. One station suddenly for no apparent reason, stopped attracting anopheles it was the only one we owned and had fully under control. Still, we have something to show for our pains two stations. There catches continued for 29 and 21 years, including the years of the last major epidemic. They indicate numerous ups and downs in the number of anopheles, but no clear case can be made out for the contention that the last epidemic coincided with an unusual rise in the number of anopheles, or that it was immediately preceded by such a rise (Tables and Graphs II and III).

There are, no doubt, some facts pointing in that direction. The Amsterdam station shows a considerable increase of anopheles in 1938, and also, although not so well marked, in 1939. Malaria in Amsterdam, increasing since 1937, showed a definite peak in 1940. Then it decreased, to rise again in 1944 to its final leap in 1946. This second rise was not accompanied by an increase of anopheles, except for the year 1946. In that year there were more anopheles than in the preceding and following year, but no more than in some of the years between 1931 and 1936, which had but little malaria to show.

The Alkmaar station showed unusually high numbers of anopheles in 1939, 1943 and 1944. Malaria incidence in that city was increasing in 1941 and 1942, coincidently with a low anopheline density. In 1946 and 1947 malaria incidence was doubled, in 1948 it was trebled. The anopheline density did not increase until 1947; in 1948 it did not reach the level of the years 1929 to 1936, which had not been remarkable for much malaria.

These few details may suffice to show that there really exist serious grounds for my doubt whether the major epidemics are caused by an unusual increase in the numbers of anopheles. But I grant that I have not offered you the definite proof that the anopheles factor ought to be excluded as an explanation of this periodicity.

There exists an alternative explanation. In Sir RICKARD CHRISTOPHERS' classical discussion of the causes of the recurrent malaria epidemics in the Punjab the anopheline factor is of undoubted importance, but it is not the only one. The human factor also contributes to bringing about this periodicity. The immunity which each epidemic leaves to the survivors protects them for a time. Then it gradually wears off, and its decline leaves the population an easy prey to the disease. The point I mentioned just now, that the anopheles may increase to numbers not above those observed in some non-epidemic years, and that this apparently unimportant increase may, nevertheless, coincide with an epidemic, would have offered no difficulty to Sir RICKARD. He would have pointed out that an anopheline density, unable to provoke an epidemic in a population still protected by their acquired immunity, may be so in a population no longer enjoying that protection.

Are these considerations applicable to North-Holland? Are we justified in assuming that epidemics affecting no more than 20 per cent of the population at the very highest estimate, could induce an immunity protecting the majority of the population for the next 20 years?

On the face of it this assumption appears absurd. Still, in 1938 I felt inclined to accept it, and for the following reasons. The first reason was the age distribution of malaria. In Wormerveer, adult malaria incidence was two-fifths of the child incidence in the years 1930 to 1936. It was not unreasonable to ascribe this to a slight degree of immunity in adults. But if this was true, and if the periodically recurring epidemics were really the consequence of the loss of this slight immunity, it followed that the difference between adults and

children should disappear shortly before or during the rise of a major epidemic. At present we know that the difference between the incidence of malaria in adults and children did not disappear before, or during the major epidemic, which culminated in 1946. It is true that the difference grew less in the years 1934 to 1942 adult malaria incidence rose to half of that in children. During the epidemic it further increased to three fifths of the incidence in children. But it never equalled it. In the town of Zaandam malaria attained a much higher figure than in Wormerveer (14 per cent. of the population in 1946). If anywhere it was there that loss of immunity would become manifest by equalizing child and adult malaria incidence but it did not. During the years of the rising epidemic (1943 to 1945) the adults incidence was half in the peak year 1946 it was three fifths of the children's, just as in Wormerveer Thus, these findings, although not decisive either way do not support the view that major epidemics in North-Holland are caused by the loss of immunity

The other reason why I once felt inclined to attach some value to loss of immunity as an explanation of the recurring major epidemics was the following In non-epidemic years malaria occurs in distinct foci. It affects a comparatively small part of the population leaving a majority practically untouched. In these conditions the true malaria incidence should be computed on the base of the population in these foci and not on the base of the whole population. Malaria incidence corrected in this way is much higher than the uncorrected one it is no longer an obstacle to the assumption that immunity can develop in the North Holland malarious areas.

Since then we have found, however that the major epidemic puts an end to the existence of these foci they overflow they flood the neighbouring areas (unaffected up till that moment) and thus they disappear for a time.

During the epidemic the malaria patients are recruited from the foci and from elsewhere. Inside the foci malaria incidence in adults probably remains at the level of the pre-epidemic period *i.e.* about two-fifths of that in children. Outside the foci adult and child incidence are probably the same Consequently the two groups combined should show a higher adult incidence but never as high as the child incidence. This, we have seen is what actually occurs. The higher adult incidence is not however the indication of a deteriorating immunity in a population too long exempt from malaria, as I supposed it is the indication of a fresh portion of the population joining the ranks of those already affected by malaria.

(2) SEASONAL PERIODICITY

Malaria in North-Holland is a disease of late spring and early summer It reaches its apex in the months of May June or July usually in July As a rule it definitely is on the decline in August. That decline is gradual, whereas the rise in March and April is steep.

We sometimes observe a second rise in September or Oct ber As a rule

this autumn peak is much lower than the spring-summer peak But in a place recently affected by malaria the autumn peak may be the larger of the two, or it may be the only peak of the year (as in the city of Haarlem, where malaria increased from 18 cases in 1946 to 118 in 1947 (Table IV) An autumn peak (even a low one) foreshadows an epidemic rise next year An early peak (in May) is followed by a decline of malaria in the next year

The spring-summer peak may be compared to the spring-peak in the Mediterranean region In that region the spring-rise is due to relapses from the preceding year In North-Holland the same applies, with this difference, however, that the greater part of these relapses originates from infections acquired in the preceding late summer or early autumn, which did not result in overt malaria They remained latent till the next spring or summer Some of them, however, became overt after the usual period of incubation If they were numerous they produced an autumn peak

TABLE IV

NUMBER OF MALARIA PATIENTS IN HAARLEM (161,450 INHABITANTS) IN 1947 AND 1948 MONTHLY FIGURES

Month	Number of malaria patients in	
	1947	1948
January	1	11
February	1	14
March	2	13
April	4	22
May	5	37
June	10	55
July	14	62
August	14	73
September	28	23
October	24	18
November	13	22
December	2	5

There is no correlation between the seasonal incidence of malaria and of anopheles These mosquitoes become numerous in human and animal habitations from the second half of July onward They remain numerous throughout the succeeding months of the summer and early autumn Their number is highest in August, September or October Then decline sets in, and a minimum is reached in May of the next year

Infected anopheles are extremely rare in late spring and early summer If at all present they are found at that time in animal habitations as well as in human habitations From the second half of August they become increasingly numerous, this time, however, in human habitations only They reach their maximum in October From then onward they gradually become less numerous,

as no fresh infections occur but they continue throughout the whole of the winter They disappear with the passing away of the hibernating generation. From January onward, however they are no longer of any importance as their sporozoites are degenerated.

All this was well known before the war I have repeated it to introduce the point I want to discuss *What is the importance of the anopheles found infected in May June and July?*

They are extremely rare at that time. Moreover in May and June anopheles are present in small numbers only Thus, I believe it is safe to say that anopheline infection in May and June is of no account whatever But in the second half of July anopheles become numerous. In that month even an infection of 1 per 1 000 or less, may be of importance the more so as the mosquitoes are flying long distances at that time of their maximal sexual activity Anopheles infected in summer are the ideal vectors for the spread of malaria over long distances. Repeating an expression I used just now Anopheles infected in summer would be the vectors suitable to convey focal malaria in the process of overflowing and flooding the surrounding area, thereby converting the focal distribution into an epidemic distribution.

If anopheles infected in summer played this part in the epidemiology of malaria in North-Holland, this would allow us properly to define the nature of the major epidemics recurring every 20 years. The definition might be something like this In ordinary years malaria is limited to more or less sharply defined foci. Inside each focus malaria is transmitted in late summer and early autumn by anopheles, found infected in great numbers at that time, which however convey the infection over short distances only

In years of major epidemics malaria continues to be transmitted by the process, described just now which we might call the "focal transmission. Moreover these years are characterized by another process which might be called "generalized transmission, carried out by sexually active anopheles in July and the early part of August. That mode of transmission is never wholly absent, but it becomes of importance in some years only If it does, it opens the way to the "overflowing of the foci which is the characteristic feature of the major epidemic.—So far for the tentative definition

But why should transmission in summer be of much importance in one year and of little importance in another To answer this question we should first answer another Why summer transmission not always important? It should be Anopheles are numerous and malaria patients are reaching their maximal numbers. The answer which still holds good as far as I can see is that numerous infected anopheles in summer are lost to malaria transmission.

(1) Because they are attracted to animal habitations when returning from the breeding places they visited for the sake of oviposition.

(2) Because their span of life is shorter than that of the sexually inactive generation (which continues to take blood) in late summer and early autumn.

My first question is modified by this answer and now runs as follows Why should malaria transmission be frustrated in the summer of some years by the circumstances mentioned just now, and not in the summer of others ? As far as I can see there are three explanations

One can be dismissed out of hand, because we do not know anything about it, the assumption that the span of life of sexually active anopheles is greatly increased in some years

The second one is of more value If there are no animal habitations, no anopheles will be lured away from the human habitations In this connection it is a significant fact that livestock, including pigs and horses, have been greatly reduced in numbers during both world wars, and that it took some considerable time to restore their numbers after the wars were over The fact that two of the major epidemics we are cognizant of coincided with the end of each world war will occur to everyone's mind as highly suggestive and stimulating But this effect will be damped by the afterthought that nothing of the kind happened to throw light on the origin of the first one of the certainly known epidemics (that of 1900 to 1902), nor on the probable one of 1880

The third explanation I have to offer is just as reasonable and suggestive, and just as unsatisfactory It is this Summer transmission is dismissed as unimportant because so few infected anopheles are found, the index of natural infection is too low But the index of natural infection is not everything, it should never be taken as an accurate gauge of the importance of a certain species of anopheles as a local vector, unless in conjunction with the numbers in which this species occurs A low index combined with huge numbers may be as important as the reverse condition

This consideration leads to the following conclusion Summer infection in anopheles, admittedly of extreme scarcity, may nevertheless greatly influence the course of malaria in some years, by causing its transmission in summer and, consequently, its dissemination outside the boundaries of the foci, *i e*, the prerequisite of a major epidemic However, it can do so only if anopheles are present in unusually large numbers And here we find ourselves back at the point we discussed before, and which we dismissed without a definite conclusion as to whether the last major epidemic coincided with, or arose shortly after, an unusual increase in the number of anopheles

One conclusion of practical importance arises from this discussion It is the following The proper time to deal with malaria in the province of North-Holland is not the period of the major epidemic, which carries away the landmarks and floods the whole country, but the inter-epidemic period, when malaria is confined to its foci Unfortunately, financial considerations are an obstacle to carrying this plan into effect The authorities who have to provide the money are ready to finance the fight against malaria when the disease makes itself obnoxious to the general public, *i e*, when it affects people to whom it was practically unknown And that is the case in major epidemics

only But when malaria retires again to its foci to that portion of the population who are well acquainted with it general interest wanes and with it the willingness to pay for malarial control.

(3) CONTROL OF MALARIA IN NORTH HOLLAND.

With the limitation imposed by the intermittent readiness to honour the bill, systematic malaria control has been going on in North Holland since 1920 It was accepted as a truth needing no proof that control by antilarval measures was not feasible in a country where the drainage system itself was the only breeding area. Later on it was found that antilarval measures were both practicable and successful but too expensive except in certain circumstances. As a consequence, from 1920 onward, malaria control consisted in destroying adult anopheles.

At first the ambitious object was to kill so many of the hibernating generation that the next generation would be substantially reduced. For that purpose the mosquitoes were killed in those winter quarters where they were more numerous than anywhere else i.e in cowsheds, stables and pigsties. Human habitations were not included, because they harbour comparatively few anopheles (tens—against thousands), and because the method of destruction, spraying a solution of lysol, was quite unsuitable for application in a well ordered household. The work was continued for 2 winters. Its effect on the anopheles population was not apparent at that time Now after so many years it strikes me that the returns of the catching stations in 1920 to 1923 were low in comparison with the catches in the following 9 years (Table and Graph II). However that may be the central government reduced the appropriation to the extent of rendering continuance along the same line impossible. That was the end of the first stage.

Attempts were recommenced in 1926 stimulated by two new findings. One was imported, the pyrethrum sprays one was home made the discovery of infected anopheles inside human habitations in autumn and winter Pyrethrum insecticides allowed of spraying human habitations. The results of the search for infected anopheles suggested a new object killing infected anopheles. This gave a promise of a more direct attack on malaria. Hence the spraying of animal habitations was abandoned and the families were exhorted to do their own spraying

Malaria control was to be carried out by the population, aided and instructed by the propagandists of the Commission for Malaria Control by the Population of North-Holland. The pyrethrum insecticides and pulverizers were sold to the inhabitants at reduced rates from the Commission's stock. The propagandists moreover kept in close touch with the local practitioners and collected their notes on fresh cases of malaria. Since the Commission started its activity a great deal more became known about the prevalence of malaria in the whole province. Eventually we lost this kind of intelligence

service Malaria now has been raised to the rank of a notifiable infectious disease That is a good thing, as it impresses both government and local practitioners with the notion that malaria is not wholly negligible, and that its diagnosis entails some degree of responsibility But less than one-tenth of the actual cases are notified Personal interviews with the practitioners remain as necessary as ever

Although successful in this side issue the Commission failed in its main object No doubt the population was actively spraying, but at the wrong time In summer they killed innumerable *Culex pipiens* But they could not be induced to continue their activity when no longer worried by the singing gnats Unless sufficient *Theobaldia annulata* were present to prompt them to further vigilance, they stopped just at the time they ought to have started, *i e*, the middle of August

Without change of name, the Commission abandoned the plan of having the population do the actual work From 1936 onward they did it by means of their own trained personnel That marked the beginning of the third stage

The Commission concentrated on malaria control in a small number of villages At that time we were still well within the inter-epidemic period, but there were villages which did not fall into step with the general course of events, which were not synchronized (as I explained before) These were the villages the Commission selected Now, 10 years later, we note that malaria was not only reduced to almost nothing in these villages, but that they remained exempt from malaria at a time when the whole of North-Holland passed through the stages of a major epidemic Nevertheless, we cannot get rid of the uneasy feeling that these villages are continuing their own independent course, and that they will start a major epidemic of their own in the late 'fifties

The new plan was based on investigations carried out in the village of Uitgeest, which were a repetition and an extension of former work They confirmed the autumnal development of anopheline infection and its almost complete absence in summer They allowed an approximation to be made as to the time required for the infection to mature about a fortnight in August and September, and 4 weeks in October They established the fact that fresh anopheline infections no longer occur after 1st November, and that sporozoites degenerate in December

The mistakes made by the population were carefully avoided. Spraying was strictly confined to the period of mature anopheline infection It started when the first sporozoites appeared (15th August), it stopped when the absence of young oöcysts announced the end of fresh infections (1st November) During that time spraying was carried out once a fortnight in August and September, and once more in the middle of October, five times altogether Per square metre (35 square feet) were sprayed 5 ml of a 1½ per cent solution of a concentrated extract of pyrethrum in purified kerosene

Not all houses were sprayed, only those harbouring infected anopheles

In the first villages which were the object of the new procedure (Uitgeest and Marken in 1936 and five villages farther north in 1938) the presence or absence of infected anopheles was determined by direct observation. Only houses harbouring infected anopheles were treated. But this method of selection had to be abandoned when the experimental stage had passed. On the other hand, the method had to be repeated too often to allow of applying it to all houses, and a selection had to be made. What would be the standard by which to judge which house to spray and which to leave?

It is only fair to admit that this was, and remained, the weak point in this method of malaria control which our American friends of the International Health Division have called "pin point spraying" In the early days we decided that the selection of houses to be sprayed could be made on the strength of the presence of at least four inhabitants under 16 years of age. It worked well in two villages, but in others it did not, and so it had to be abandoned.

In view of the great importance of subclinical malaria as a source of anopheline infection our standard is at present all houses are to be sprayed which are inhabited by persons who had malaria in the current year or in one or both of the preceding years.

It would be easy to apply this standard if we knew who had been suffering from malaria in the current or preceding year But often we do not know and so our knowledge must be supplemented by the result of spleen examination blood examination and local enquiry before the spraying list can be made out for the village under consideration, i.e. the list of houses to be sprayed when the time has arrived to do so. In towns, more than in villages, this does not always work well, and one often feels as if one were working blindfolded. In any case our method definitely prevented our work from proceeding at any but a very slow pace.

That was the condition we were in when DDT appeared on the scene and the Rockefeller people advised us to abandon pin point spraying altogether They even advised us no longer to specialize on house-spraying and to include animal habitations in our programme. As far as house spraying was concerned, the following of this advice was well within the range of possibility If we did not wish to go in for species eradication, one operation in each house was sufficient to keep it free from anopheles, not only during the period of anopheline infection (August, September and October), but also during the time when summer transmission may occur (July). Spraying in June and the first half of July would prevent both.

The introduction of DDT in our programme of spraying (1945) marks the beginning of the fourth stage of malaria control in North-Holland. In one village only this included wholesale spraying in all others pin-point spraying continued. The municipal authorities of Amsterdam have applied wholesale

TABLE V

ACTUAL NUMBER OF MALARIA PATIENTS IN VILLAGES TREATED BY SPRAYING SELECTED HOUSES WITH PYRETHRUM, FIVE TIMES IN AUGUST, SEPTEMBER AND OCTOBER.

Years.	Uitgeest. Number of patients (2,901 inhabitants).	Marken. Number of patients (1,470 inhabitants).	Grootebroek and three others. Number of patients (9,201 inhabitants)	Wervershoof. Number of patients. (2,414 inhabitants)
1927	59	40	38	6
1928	32	26	65	17
1929	18	26	109	8
1930	73	33	106	21
1931	[illegible]	17	85	4
1932	47	6	1	1
1933	124	33	7	0
1934	179	103	2	1
1935	629	63	7	12
1936	414	152*	82	69
1937	85	10	82	65
1938	21	1	923	118
1939	10	0	298	35
1940	0	0	103	16
1941	5	1	10	
1942	0	0	0	0
1943	12	6		1
1944	9	1	0	1
1945	3	2	5	0
1946	10	2	1	0
1947	8	5	1	0
1948	8	2	0	0
1949	1	6	0	1

* Sprayed for the first time.

N.B. To check the results, Tables I to III and Graph I may serve as control

TABLE VI

ANNUAL NUMBER OF MALARIA PATIENTS IN VILLAGES SPRAYED ONCE WITH DDT IN 1947 AND IN UNTREATED VILLAGES (OR TOWNS)

Year	Number of patients in		Number of patients in	
	Alkmaar Not treated 39,240 inhabitants	Helder Not treated 33,408 inhabitants	Zaandam Treated (pin-point) 43,083 inhabitants	Wormerveer Treated (pin-point) 10,000 inhabitants
1943	391	13	61	54
1944	310	15	315	275
1945	331	23	1,823	332
1946	604	35	6,024	696
1947	650	276	1,586	305
1948	1,084	1,450	242	21
1949	65	118	52	7

Year	Number of patients in		
	West Zaan Not treated (3,320 inhabitants)	Oost Zaan Wholesale spraying (4,303 inhabitants)	Wormer Pin-point spraying (4,911 inhabitants)
1943	3	20	21
1944	20	41	44
1945	79	129	188
1946	249	349	705
1947	94	76	261
1948	31	5	27
1949	1	2	5

Year	Number of patients in Amsterdam	
	North of river Y, Wholesale spraying in 1946 and 1947	South of River Y Not treated
1943	230	21
1944	718	57
1945	779	83
1946	2,090	326
1947	242	78
1948	38	95

DISCUSSION.

The President I think there was no need for the concluding words of Professor SWELLENGREBEL. You have listened, I think, to a scientific paper delivered as a scientific paper should be. Professor SWELLENGREBEL's treatment of his material has been the essence, the distilled essence perhaps I should say of the scientific method. Time and again he produced attractive and apparently significant reasons for the prevalence or absence of malaria in one place or another and at one time or another only to demolish the evidence and bring us back to the position of Wait and See. We can draw from that the deduction that any conclusion of Professor SWELLENGREBEL is a well and truly proven fact.

Professor G Macdonald What a pleasure it is to listen to Professor SWELLENGREBEL. I have had the privilege of listening to him three times, and have been more fascinated with the clarity and fairness of his exposition on each occasion. This paper is very stimulating he produces evidence for the occurrence of a regular periodicity in the incidence of epidemic malaria in Holland, and has failed to demonstrate any increase in anophelism, or other obvious causative factor to account for the individual epidemics or for their periodic occurrence.

Periodicity is no new thing in epidemiology The notorious malaria epidemics of the Punjab and of Ceylon showed it, but in their case there was, in addition to the cyclical factor which CHRISTOPHERS attributed to the waxing and waning of immunity an anopheline factor which obviously resulted in increased transmission. Periodicity is also well known in the epidemics of other diseases, particularly those which confer some lasting degree of immunity and has been reproduced in experimental animals (GREENWOOD 1932), and explained mathematically (SOPER, 1929) on the basis of a general theorem of epidemics elaborated by ROSS (1915) and ROSS and HUDSON (1917). In these cases periodicity is quite independent of any obvious factor which might be expected to increase transmission at the epidemic times, or of any change in the virulence of the organism concerned, and is solely due to predictable changes in the number of susceptible persons in the population. Can the explanations which satisfactorily explain cycles of these other diseases be legitimately extended to cover the case of malaria in Holland? And if so, what general degrees of infectivity and immunity would be needed to produce the cycle actually demonstrated?

To simplify perhaps over-simplify matters, it seems that the interaction of three factors are responsible for epidemic rises and periodic cycles of epidemics in the absence of any change in virulence of the virus or ease of transmission. The interacting factors are the reversion time or period after infection before the individual again becomes susceptible the incubation interval or period between infection of one case and the development of a

similar state of infection in secondary cases, and the infectivity or number of secondary cases normally infected from an original one in unit time

The reversion time is probably very long in Holland, where there are very few strains of parasite, the incubation interval seems very long and, indeed, the data presented suggest that it may often be a year Under these conditions periodic cycles would seem from SOPER's analysis to be very likely, and not to demand changes in ease of transmission Mathematical calculations based on them can only be very rough approximations, but it does seem from an analysis that an infectivity of something between 1 4 and 2 7 a year might well produce the endemicity and periodic epidemics actually observed *

These figures are of the order that is to be expected in Holland, and they, therefore, do not contradict the hypothesis that epidemics are caused by factors independent of variations in anophelism Is there any positive support for it? If it were correct one would expect, on looking at an annual graph of cases, to see, not a long plateau with an occasional steeple of an epidemic but some definite evidence of wave action, a building up of cases in several successive years to an epidemic peak The tables in the paper give evidence about twelve epidemics in Wormerveer in 1902, 1922 and 1946 (Table I), Amsterdam in 1922 and 1946 (Table II), Alkmaar in 1948 (Table III), Uitgeest in 1935, Marken in 1936, Grootebroek in 1938, Wevershoof in 1938 (Table V), Helder in 1948 and Zaandam in 1946 (Table VI) In the case of two there is no information about the previous years, in all the others there is, in fact, a slow build up over 2 or 3 years to constitute the epidemic wave

The figures were not collected for the purpose, and in any case need analysis by a much more competent person than myself, but they are at least compatible with a natural recurrent periodic wave, such as that which occurs in measles, which does not demand the prevalence of excessive numbers of anopheles, or extraordinary conditions of transmission for the production of epidemics, although undoubtedly these waves cannot occur unless conditions are favourable, and would naturally be increased when conditions were particularly favourable I put out that suggestion with considerable hesitation in the presence of the authority who has just talked to us, and who had doubtless considered it

The point arises, if this suggestion has some grain of truth in it, what bearing has it got on the question of control? Well, it conforms with the conclusion that Professor SWELLENGREBEL has arrived at, that the time to

* Assuming the incubation interval to be one year, and the known periodicity being 22 years, the s of Soper's $2\tau\sqrt{s\tau}$ is 12 3 years This corresponds to 2τ in Ross and Hudson, who give the value of τ as $\gamma R\tau = \log_\epsilon P_0 + \log_\epsilon \gamma - \log_\epsilon (\gamma + 1)$ Assuming the above value of τ, a reversion factor of 90 per cent in 10 years and no mortality from malaria, $\gamma = 5$, and the infectivity $c = D + (\gamma + 1)R = 1\ 4$ This working is intended as a rough example to show the order of events only

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endemic villages the size of the average enlarged spleen is significantly greater than elsewhere, and they can be picked out with ease in this way

With regard to the control of malaria in Holland, I have always been interested in measures for the destruction of the infected adult mosquito. In a lecture given yesterday at the London School of Hygiene, Professor SWELLENGREBEL mentioned that SCHÜFFNER had taught his assistants in Java to swat mosquitoes in dwellings where fever cases had occurred. LE PRINCE had adopted the same procedure in quarters occupied by labourers in Panama during the construction of the Canal, with good results as regards malaria incidence. S. P JAMES also advocated an attack on the adult mosquito as a major method of malaria control. Professor SWELLENGREBEL was a pioneer in the spray-killing of adult mosquitoes, a method which with the development of residual insecticides has achieved such remarkable results in many countries. I think I am correct in stating that his work in Holland inspired the campaign in South Africa, which was the first example of a successful attack on rural epidemic malaria by this or any other method. I mention this to draw attention to the fact that Professor SWELLENGREBEL is not only a great epidemiologist but also a pioneer in the development of one of the most effective methods of malaria control.

Professor R M Gordon I would like to add my tribute to Dr SWELLENGREBEL to those of our President and Professor MACDONALD Colonel SHORTT has referred to Professor SWELLENGREBEL's address as a distilled essence. I would add that the drink has been flavoured with humour also that it is an exhilarating one so that one must be careful, as Professor MACDONALD pointed out, not to offer facile explanations of a complex problem. I will confine myself to asking Dr SWELLENGREBEL two questions (1) I am not sure to what extent he thinks it probable that summer infections amongst anophelines showing a very low infection rate, but a very high density may have resulted in the dissemination of malaria outside the boundaries of the foci he has described to us this dissemination being the essential precursor of a major malaria epidemic. Am I right in concluding that he thinks this was probable but that he lacks the necessary data to prove or disprove it, because although he knows the density of the anophelines collected during the inter-epidemic periods at the stations outside the permanent foci he does not know the infection rate amongst them? I am very glad Professor SWELLENGREBEL has referred to "anopheline infective density Some 15 years ago Professor DAVEY and I when working in West Africa, drew attention to the fundamental importance of anopheline infective density and since then we have come across many instances of failure to grasp its significance. Thus, last year a paper was published concerning malaria in the Belgian Congo and it was stated that the main species of anopheline was *A moucheti* and that there were some nine *A moucheti* to every one *A gambiae* captured in the houses. But the

writer's dissection results showed that the infection rate amongst *A moucheti* was 0 4 per cent., and amongst *A gambiae* it was 4 0 per cent, so that on his own showing *A gambiae* and not *A moucheti* was the more important vector Incidentally, I am only criticizing the interpretation of the figures presented in this preliminary paper, for I believe that further work proved that the author was correct in believing *A moucheti* to be the more important vector

(2) Professor SWELLENGREBEL mentioned that in malaria epidemics in North-Holland the most marked rise in malaria occurs amongst the adults in the human population, but that the marked difference between child and adult infection rates noted during the inter-epidemic periods does not disappear, as it might be expected to do if the rise in the incidence of malaria was due to a loss of immunity in the adult population I would like to ask Dr SWELLENGREBEL if he has any records of the infection rate amongst infants in the area, amongst whom the question of acquired immunity does not arise?

Mr P G Shute Although Professor SWELLENGREBEL has spoken only of malaria in Holland, it occurred to me that it may be of some interest to say a few words about malaria in England in recent years I cannot speak of endemic malaria in England because, as far as is known, it doesn't exist, but we do have sporadic cases of indigenous malaria, by which is meant cases arising from imported gametocyte carriers and transmitted by our indigenous anopheles mosquitoes Our climate is much the same as that of Holland, and we have the same insect host, *Anopheles maculipennis atroparvus*, I think as numerous, at least locally

Nearly 25 years ago I had the privilege of showing Professor SWELLENGREBEL around a small village in Kent where, between 1917 and 1921, there were about 100 cases of indigenous malaria (all B T) out of a population of 400 inhabitants This village, Grain, is situated on a so-called island at the mouth of the River Thames, only 50 miles from this hall There are only three farms there, but many of the villagers keep pigs, rabbits and poultry, or did at that time In the farm buildings and pigsties thousands of atroparvus are present during the late summer months, and I have on many occasions collected 2,000 and 3,000 in a few hours

Just across the River Medway is situated the Isle of Sheppey, and further round the coast to the east is the town of Sandwich In both of these areas there were quite a large number of indigenous cases of malaria between 1917 and 1921, so that between these years there were 384 cases notified in the county of Kent

In the whole of England during this period (1917 to 1921) there were 481 cases of indigenous malaria Twenty-one counties contributed 87 cases, and one, Kent, 394 If it were that in these areas in Kent the atroparvus density greatly exceeded that of all the other counties, a simple explanation for the little epidemics in Kent might be conjectured, but many parts of the south

and south-east coast, including Suffolk, Essex, Sussex, Hampshire and Dorset, abound with atroparvus which are as numerous there as they are in Kent. It would seem that during the years 1917 1918 and 1919 the percentage of the population in the villages of Grain, Queenboro and Sandwich infected with malaria was as high as it was in Wormerveer Yet the disease died out spontaneously if we except quinine treatment of the overt cases and some small scale anti larval operations.

Following the second world war and the return of numerous troops who were potential relapsing cases, as well as numerous Polish refugees, one or more cases of indigenous malaria occurred in villages in 12 counties, but this time there were not more than three in any one county including Kent.

It is, I think, difficult to understand why the disease disappeared so abruptly after 1919 in those villages where 10 and even 20 per cent. of the local population were infected. It is equally difficult to understand why there were so few cases following the second world war Puzzling too, I think, is the fact that we so often have a single case of indigenous malaria in a village. We know that a reasonably heavily infected mosquito can, providing it lives long enough (about 30 days after it becomes infective) infect 15 patients. One would have expected, I should have thought, that there would usually be three or four cases instead of just one. It is interesting to record that during the past 30 years one or more cases of indigenous malaria have occurred in more than half the counties of England, from Yorkshire to Devonshire (26 to be exact), and one in Northern Ireland. Professor SWELLENGREBEL says there is no doubt that in his country there exists a periodicity in the occurrence of major epidemics at periods of 20 to 25 years. I wonder does he think that this could happen in the absence of a low degree of endemicity? Except in special circumstances it would seem that there cannot be an epidemic of malaria in the presence of hyperendemicity or in the absence of low endemicity In present-day England it would seem that even when 5 to 20 per cent. of the population become infected in an area where the anopheline density is high, this is insufficient to produce an endemicity of long duration. During the past 2 or 3 years I have been working with *Anopheles quadrimaculatus* surely a first cousin of atroparvus, and I am convinced that it is much more anthropophilic than is atroparvus, and I wonder whether if this species were indigenous we should have endemic malaria. I should be glad if Professor SWELLENGREBEL would give me his views.

I wonder if the bionomics of anopheles which transmit malaria in Holland may at least in part, help to explain the much higher sporozoite rate between the latter half of August and November as compared with the early part of the summer June to the middle of August. During early and middle summer atroparvus, or as Professor SWELLENGREBEL prefers to call them, short wing maculipennis, are very fully occupied with reproduction. This is an intense concentrated and dangerous struggle, and it is very doubtful if many survive

for more than a week or two A single blood meal matures the ovaries in from 48 to 96 hours, and this cycle continues for many weeks if the mosquito survives Every 2 or 3 days it has to leave the place where it obtained its blood meal and find somewhere to lay its eggs Having achieved this, it then returns to its old haunt, or a different one, and the whole process begins all over again

It is generally accepted that atroparvus is a zoophilic parasite, but this is not necessarily true for the whole of its life if it happens to survive into the late autumn It depends on where it happens to be when nature intervenes in late summer and the process of gonotrophic dissociation sets in When this happens with some species of mosquitoes, they immediately seek dark, cold resting-places where they can hibernate for several months, but this is not the case with atroparvus Although the females are sexually liberated, they continue to take blood meals throughout the winter, how frequently depends on the rate of digestion of the previous blood meal, which is governed by atmospheric temperature If, therefore, gonotrophic dissociation sets in when the insect is in a pigsty, it will remain there for months and only leave voluntarily if the pig is taken away On the other hand, if the mosquito happens to find itself in a human habitation when gonotrophic dissociation sets in, then it will remain there for weeks or months and become a permanent member of the family, just as much as a dog or cat—in fact, more so, because it isn't turned out of the house either by day or by night

One can therefore say that there is a period in the life cycle of atroparvus when it may be completely zoophilic on the one hand, or, on the other hand, completely anthropophilic, depending entirely on where it happens to be at the time when ovary development ceases

It is these sexually liberated atroparvus which, having taken up residence in human habitations, may change almost overnight from harmless insects to highly dangerous ones A few atroparvus entering a house in late August where there are several children, and one of them being infective, these mosquitoes may quite well transmit the disease to every member of the family The children would probably be infected first, even should the parents escape, because usually the children go to bed several hours before the parents, and so hungry mosquitoes would feed on the children before the parents retired for the night

I should also like to say a few words about true B T latent malaria Professor SWELLENGREBEL says that in the Mediterranean region the spring rise is due to relapses of the preceding year, and that in North-Holland the same applies, except that the greater part of these relapses originate from infections of the preceding summer or early autumn which did not result in overt malaria But should such cases come under the category of relapses? Even with our Madagascar strain, long-term latency is not unknown We believe it has something to do with the quantum of sporozoites About half of the 520 cases

of indigenous malaria during the past 30 years which have been investigated have been cases of true latency

True latency in B.T malaria is it seems to me a phenomenon which occurs where there is an absence of a *pathogenic condition and erythrocytic invasion* by the parasite over a period of several months, with an average of 38 weeks and a maximum period of about 1 year It may occur in several ways

(1) *As a natural event*

In nine carefully investigated cases the average incubation period was 282 days.

(2) *Following drug prophylaxis such as mepacrine or pamaquin, but not quinine the drug given 1 day before infection and for 3 or 4 days after infection.*

Nineteen selected cases showed an incubation period of 263 days.

(3) *Immune to one strain clinically and parasitologically and infected with another strain.*

Six selected cases showed an incubation period of 284 days.

(4) *Long-term relapses following the primary attack which was treated by anti malarial drugs.*

In 52 cases the average number of days from the primary attack until the relapse was 263.

Although it is perhaps highly improbable, it almost looks as though there is a stage in the life history of many strains of this species of parasite which takes about 38 weeks to complete its life cycle before becoming pathogenic. If this is not so, then it would seem that there must be a long resting phase of the parasite not, presumably associated with erythrocytes.

Two years ago we had a practical demonstration of true latency with the Holland strain of B T Two girls, aged 15 and 16, went to Amsterdam f r a holiday in 1946 with a group of other girls from their school. Both live in a rural part of Surrey where maculipennis are prevalent. One of the girls was taken ill in January 1947 and the other in May of the same year and B.T malaria parasites were found in their blood. Not unnaturally the M.O H reported them as cases of indigenous malaria but, on investigation it was obvious that they were cases of true latency Neither of them had had any illness for over 1 year

This problem of long latency in benign tertian malaria is, at present difficult to explain and the time factor of the two groups reported bove—latency produced as the result of drug prophylaxis and long-term relapses—both groups *having an average incubation period of 263 days, suggests,* I think, that there is a common factor between them.

Colonel E L. Perry I do not think I can add very much to this discussion but I will say this, that I came up especially from Devonshire t hear Professor SWELLENGREBEL. I have met many distinguished malariologists—LAVERAN MANSON ROSS, MESNIL, of the Pasteur Institute, and many amongst my own

colleagues, and now I have had the pleasure and honour of listening to Professor SWELLENGREBEL, of whom I have known for many years It is nearly 40 years since I made any contribution to our investigation of malaria, and certain things have struck me in Professor SWELLENGREBEL's talk I am glad that he now talks freely of immunity in malaria, because when I first raised the question of there being immunity following the epidemic of 1908 in the Punjab I was torn to pieces by statisticians who said that people who had malaria in the blood could not be immune After that great epidemic in 1909 the population of the affected areas had parasites in their blood in enormous numbers, and in the autumn all other factors favoured malaria, but there was no epidemic I should like to ask Professor SWELLENGREBEL whether he has considered the relationship of nutrition to malaria? The point did not come to me in 1909 until I heard SIR RICKARD CHRISTOPHERS' suggestion that the culmination of an epidemic was due to malarial conditions occurring after a drought had produced very considerable malnutrition amongst the population I would ask Professor SWELLENGREBEL if he has considered the question of the introduction of malaria into the villages by persons returning from the Dutch tropical possessions? Also whether he made any particular observation on the relative prevalence of the species of malaria, falciparum, etc, and whether there is any quartan? Although Professor SWELLENGREBEL is so very modest in saying that he has not proved any very great conclusion by his work, I will say that by what he has done, with the sparse material at his disposal, he has definitely proved the truth of what the poet wrote that "No unregarded star contracts its light into so small an amplitude, but if we steadfast look in it we may discern, as in some sacred book, how man may heavenly knowledge learn"

Dr P C C Garnham In the rather cloistered environment of North-Holland it would seem not unlikely that a very small number of strains of *P vivax* are concerned in malaria In such circumstances, the immunity following attacks of this disease is likely to be stable and lasting Is the existence of such an immunity capable of direct experimental proof? It seems as though the answer may already be provided in the records of malaria therapy for neurosyphilis in North-Holland Do these records show the existence of (*a*) an unusual resistance to infection in G P I cases during the inter-epidemic periods, and (*b*) ordinary susceptibility at the end of these periods? Provided that the strain of *P vivax* used in therapy were a local one, useful evidence along these lines regarding immunity (and the explanation of periodicity) might be forthcoming

Dr Muriel Robertson I should like to ask Professor SWELLENGREBEL if the intensive treatment now practised with modern drugs has done anything to lower the infection rate in mosquitoes? Populations are much more intensively treated today than in the past, and that must, one would think, exercise some influence on the number of mosquitoes carrying the disease

Professor Swellengrebel (in reply) You know that Holland has been isolated from the Western world during the throes of the German occupation, and it is only recently that we have been able to rebuild connections with the West as I pointed out at the Washington Congress of 1948. The discussion with which my paper has been honoured tonight clearly proves how very important it is for us from Holland, and more especially for myself, to come again into close contact with scientists from the West and I am grateful to all who have contributed to this discussion.

Professor MACDONALD has been too modest he was perfectly right to enter into the subject with an unwarped sense, seeing things which I myself did not see, and which I wish he had allowed me to see more clearly now He especially directed attention to the major epidemics not occurring immediately but that they are built up step by step I must say that I have not quite followed his reasoning, but I expect to do so later on. I am sure some explanation may exist which I have overlooked, and may eventually indicate a solution of the problem of the periodicity of malaria which we in Holland have not seen yet. I am glad, however that one of his conclusions was to confirm my view that the attack on malaria ought to be made during the inter-epidemic rather than the epidemic period. I am sure that his views on this matter will encourage the health authorities in Holland to continue their efforts to combat malaria just in this critical period in which we are now when malaria is going down and everybody says, "It is over we can spend our money on something else. For that reason, especially I am extremely grateful for what Professor MACDONALD has said.

Sir GORDON COVELL's initial remarks, kind and appreciative though they were, conveyed an unintentional reproach of ingratitude for a man whom, for some inexplicable reason, I did not mention—Colonel S. P JAMES. I had been in touch with him since 1920. I was influenced by his book, "*Malaria at Home and Abroad*, which he gave me, autographed, that year All I can say now is that, if we in Holland were pioneers (as Sir GORDON says) we became so as JAMES's pupils.

Sir GORDON COVELL has asked some questions which are highly relevant but not easy to answer First of all, he asks whether during the major epidemics there is not only a greater number of cases of malaria but if every single case is more severe. Although we have only to deal with benign tertian malaria, I think I can answer that question in the positive During a major epidemic a large number of people are attacked who have never had malaria before These persons begin their illness with the initial remittent fever" we associate with KORTWEG's name, and which lasts from 3 to 5 days. The number of parasites found in that condition is very small, and quinine does not control the fever Eventually there comes a true intermission, followed by the first real paroxysm and a second complete intermission the number of parasites increases and the case becomes amenable to quinine In persons

who have had malaria before there is no such initial remittent fever quinine controls the fever from the very beginning Moreover, the initial remittent fever is not so readily recognized as malaria so the specific drug may be withheld and the illness unduly prolonged

His second question relates to the increase of the span of life of the anopheles during epidemics Sir GORDON has shown that in the severe epidemics in the Punjab and Sind conditions prevail which temporarily increase the longevity of anopheles I do not know any clear instance of such a thing happening in North-Holland I realize the great importance of the question but I must leave it unanswered

The third question deals with the reduction in livestock I have pointed out that the last two epidemics each occurring shortly after a world war, were accompanied by a marked reduction in livestock, more especially pigs and horses Pigs and horses, in sties and stables attract immensely large numbers of anopheles during the summer If pigs and horses are reduced greatly in number that very potent attraction becomes much less But our difficulty is with the epidemic of 1902 It did not come shortly after a world war The question arises was there a reduction in the number of pigs and horses shortly before that epidemic I went into that question rather carefully when I wrote my report on malaria for the League of Nations Malaria Commission in 1924, and I concluded that, so far as that first well-known epidemic is concerned there is no evidence that there was any marked reduction either in livestock in general or in the number of horses and pigs, so this factor cannot explain the occurrence of the 1902 epidemic

Sir GORDON's fourth question (why did I not mention the name of the species of anopheles I referred to in my paper) can be answered as follows In Holland we have discovered two subspecies of *A maculipennis* They are sexually isolated from one another, but we have always considered them in relation to conditions in Holland only therefore we always called them "short wings" or "long wings," without asking whether they are in any way related to anopheles of other countries Entomologists have identified our "short wings" as *A atroparvus* and our "long wings" as *A messeae* So I had the choice of referring to my anopheles as "short wings" or as "atroparvus" "Short wings" would have meant nothing to most of you, "atroparvus" would have suggested that our "atroparvus" are the same as in Portugal or Hamburg, so I thought it was better not to mention any name at all But now that Sir GORDON asks the question I can say that the anopheles I mentioned were *A atroparvus* because in the salt or brackish water of North-Holland *A messeae* is in a very small minority Finally, there is the catching of adult mosquitoes by LE PRINCE in Panama, as a highly efficient control measure I am really sorry that I did not mention LE PRINCE's very notable merit when I discussed the subject yesterday with the students of the School, and I am grateful to Sir GORDON for reminding me of it

Now I come to Professor GORDON's questions. There were so many subjects he referred to that I may have missed some One related to numerous anopheles being infected to a low degree as compared with many fewer anopheles infected to a high degree. I agree with Professor GORDON that an anopheles present in large numbers, but infected to a low degree may be even more important than another anopheles infected to a much higher degree but present in smaller numbers. I think a marked example is the *A. punctulatus* group in New Guinea. Often these anopheles are infected to a low degree but are present in such immense numbers that there can be no doubt about their being the vectors which cause that high degree of infection of the indigenous population which often leads to conditions more or less like some in Africa. As to infection rates in Holland, we know them in years of major epidemics. I may refer him to various publications on the subject which show that the infection rate of the anopheles found in human habitations is extremely high in the infective season over 15 per cent. But what he is really interested in, as I can very well understand, is what is the infection rate in the inter epidemic years ? I must confess that I do not know We ought to have continued our work on anopheles infection not only during the few years of the major epidemics, but also in the inter-epidemic period. But we have not done that, and it is a very serious gap in our knowledge. Another question he asks is whether there is a higher incidence in infants as distinct from children ? There is not. When computing the incidence of malaria in succeeding age groups, we find a low incidence of malaria in infants. It rises in the second year of life remains high with ups and downs during child life and then drops to a lower level in adult life. The infection rate of the adults is lower than that of the children, but higher than that of infants. The higher incidence of malaria in children (infants excepted) was for me an indication—although not a proof—that there exists a certain degree of immunity in the population of North-Holland.

Mr SHUTE has told us of the very curious fact that in the second world war there was not that rise of malaria observed after the first, and which in some places at any rate was as high as ever we found it in North-Holland. He also pointed out, and I quite agree with him, that the part of the atroparvus population which is inhabiting human habitation becomes purely anthropophilic from the moment it stops oviposting but continues to feed, whereas the other part, which at the time of gonotrophic dissociation, is inhabiting animal habitations, becomes purely zoophilic.

Colonel PERRY points out that I have been making use of the word immunity rather loosely I am not at all sure that I had a right to use the word immunity I ought to have used the word tolerance or premunition and the only reason I used the word immunity was because I wanted to make myself clear He asked a very pertinent question, has nutrition anything to do with the origin of the major epidemics ? The fact that two epidemics occurred after world

wars is very suggestive of the idea that nutrition had something to do with it —especially after the second world war Some of you know that if the Royal Air Force had not dropped food over North Holland we might all have starved We were very badly off, and it is not at all impossible that that may have contributed to bring about the epidemic which culminated in 1946 The only difficulty in accepting that very alluring explanation is the existence of the major epidemic of 1902 there was nothing wrong with nutrition in the years preceding that Otherwise I would be quite happy to accept the suggestion He also asked about the epidemic being helped to grow by the introduction of foreign strains of plasmodia from the tropics In the years when the 1946 epidemic was growing there was no contact at all between our country and Indonesia There could not be any introduction of foreign strains, and the same holds for the epidemic that culminated in 1922 It is true that after our liberation, and after the Japanese capitulation in Indonesia, large numbers of Europeans returned from the tropics, and a great number of them relapsed, usually with vivax malaria In Holland relapses of falciparum in people returning from the tropics are much rarer The first of these people returned to Holland in 1946, at a time when the epidemic had almost reached its maximum Had they returned in 1943 or 1944, that might have explained the epidemic, but they came back too late As to other species of plasmodia, there is quartan malaria in North-Holland, but it is so extremely rare that I cannot help feeling that it does not make any difference whether it is there or not They say that in former years there was a great deal of quartan malaria in the province of Zeeland, and it is possible that there is still some left But I only know of one such case and most likely that was imported from somewhere else

Dr GARNHAM has mentioned a point I had included in my paper, but I crossed it out It is a very important question, the scarcity of vivax strains in Holland I cannot prove it, but I am sure Dr GARNHAM is right when he supposes that there are very few vivax strains in our country Probably there exists only one characterized (1) by its tendency to long incubation, (2) by its comparative resistance to neo-salvarsan, and (3) by the small numbers of merozoites No more than an average of 12 per sporulation as was found by DE BUCK We have never found any other strain in Holland, and it is quite possible that there is only this one in the whole of the Netherlands If that were so, it would certainly favour the production of immunity In G P I patients it produces immunity which lasts for a considerable time If the patient has been treated with vivax malaria and returns after a year, or even 2, or in some cases 3 years for a new treatment, he cannot be helped Infection is possible but he does not respond to it Dr GARNHAM has also asked whether there is evidence that immunity really exists by the fact that a number of persons cannot be treated successfully for G P I with the malaria cure, as is the case in Java, Rumania, and other malarious countries I know of no observations to that effect in Holland

Finally Miss ROBERTSON asks me whether the new anti-malarial drugs have not influenced the rate of infection by anopheles. I can give her only one answer and it is an answer she probably knows. In the village of Wormerveer from 1933 onwards, all malaria patients have been treated with the combined quinine plasmoquine (pamaquin) cure. For children the doses are reduced, but for an adult person the dose is quinine sulphate gramme 1 and plasmoquine mg 30 daily for a fortnight. At first the local practitioners gave quinine plasmoquine for a week, and they did not see any reduction in the rate of relapses, but if the treatment was continued for a fortnight the number of relapses was reduced to a considerable extent. That was a confirmation of the results obtained by SINTON in India. That has become a routine in Wormerveer We did not notice any appreciable difference between the rate of infection by anopheles in Wormerveer as compared with the rate in the neighbouring village of Uitgeest, where no plasmoquine was given besides the quinine, and where the relapse rate was not reduced. We have no experience of the modern antimalarial drugs. Paludrine (proguanil) has been tried in Wormerveer by Dr KLOPPER as an experiment it has given satisfactory results, but it has not become a routine method for the treatment of malaria. I cannot tell you anything about the infectivity data in relation to treatment with this drug.

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COMMUNICATIONS

A SURVEY OF SIGNS OF NUTRITIONAL ILL-HEALTH AMONG THE AZANDE OF THE SOUTHERN SUDAN *

BY

P H ABBOTT, M B, B CHIR,
Sudan Medical Service

It has long been appreciated that the nutrition of most tribes in the Southern Sudan leaves much to be desired, but the extent and severity of nutritional deficiencies is unknown, though periodic famines occur over wide areas at certain times of the year

The Azande† are probably the best nourished tribesmen in the two Southern provinces Their country enjoys a rainfall higher than elsewhere (an average of 1,438 mm per annum at Yambio over the last 10 years), and the land is for the most part fertile Famine conditions are never seen Some of the older men, it is true, can remember famines and bad years, but the introduction a long time ago of manioc as a famine crop has provided a source of food which never fails The country is well wooded with numerous permanent streams, and many of these, particularly in the western part of the area, bear narrow fringes of Gallery Forest Game, at one time believed to be plentiful, is now relatively scarce and the prevalence of the tsetse-fly prevents the keeping of cattle except in Government stations

The Azande were chosen as the subjects of the first detailed dietary s in the Sudan because of a far-sighted development scheme upon whic

* I wish to thank Mrs G M CULWICK for her assistance and for her pre notes on the dietary survey My thanks are due also to the Director, Sudan Service, for permission to publish this report

† Sing Zande Plur Azande

Government has embarked. Briefly the scheme is concerned with the development of the internal economy of the tribe, based on the growing spinning and weaving of cotton, the production of oils, soap, etc. to a point where it can support a higher standard of social, educational and medical services. As a preliminary to controlled cotton growing it is planned to resettle the whole population in permanent village lines and already (1949) the greater part of resettlement is complete. Each family has a plot of approximately 40 acres, and it is hoped that this will provide a sufficient area of cultivation and fallow to support the family indefinitely and allow for an acre or two under cotton. Workers attached to the factories of the scheme will live in rural settlements, each with a smaller plot of land, adjoining the factory sites. A knowledge of the nutritional background of the people is of the utmost value in determining future policy and indeed in ensuring the welfare of the native workers in the industries of the scheme itself.

Mrs. G. M. Culwick came to the area in June, 1947 and for just over a year was busily engaged in collecting qualitative and quantitative data on the diet of the people. The clinical survey which forms the main subject of this report, is complementary to her more detailed and arduous work, for in the words of Platt (1947) "... a satisfactory measure of its [the nutrition problem's] nature and dimensions can only be obtained from surveys for evidences of nutritional ill health, preferably combined with a study of food consumption, of food supplies, and of various factors likely to affect the food economy."

It is hoped, too, that apart from the local value of this survey a comparison and correlation of the physical signs of nutritional ill health found in this tribe with the figures for their dietary intake will be a useful contribution to the growing body of comparative data from which will ultimately be answered the vexed questions regarding the significance and specificity of accepted signs of nutritional deficiency.

Mrs. Culwick has supplied a summary of the essential dietary data, and this is given in Tables III and IV with a preliminary note. Fuller details and a description of the methods used must await the publication report.

The Method of Clinical Survey

Every month all the members of one of the chiefships ... ub-chiefsh... inspected for cases of sleeping sickness. One ... bject are together in one place and the neck glands ... absentee rate is seldom more than 4 per ... inspections during the months of October ... taken at random and examined for ... were taken in each of five groups, but apart with frank and obvious disease such as k...

fair to assume that the samples were representative of the chiefships as a whole

In addition to surveys at these inspections, boys and girls at the Church Missionary Society school, and families of the police at Yambio, were examined The groups chosen were as follows

Group I	Boys aged c 4 to 16 years
Group II	Girls aged c 4 to 16 years
Group III	Adult men
Group IV	Adult women
Group V	Pregnant women and nursing mothers

Groups I, II, and V , the young and the maternal, were taken as being most likely to show signs of nutritional ill-health owing to the strains imposed by growth and maternity

The chiefships, the number in the samples, and the months in which they were examined, were as follows

Chief	*Year of resettlement*	*Month of examination*	*Number examined*
Gangura	Second year	January, 1948	107
Ukua	Second year	December, 1947	69
Zungumbia	First year	October, 1947 February, 1948	96
Sangba I	First year	November, 1947	55
II	Not resettled	November, 1947	100
Mission school	—	December, 1947 April, 1948	108
Police families	—	February, 1948	68
		Total	603

Persons for examination were examined systematically for the signs chosen whilst a clerk noted them down Boys and men were clad only in shorts, usually of bark-cloth, whilst the majority of the girls and women wore only a bunch of leaves fore and aft No attempt was made to examine the scrotum or genitals as this would certainly have made the people unco-operative

Description of the Signs

One of the difficulties in a survey of this kind lies in the appreciation of the signs themselves The observer in his figures states categorically that a certain sign is present or is not, whereas in many conditions there is a gradual transition from the normal on the one hand to the grossly abnormal on the other, and the point at which the normal ends and the abnormal begins is vague and ill-defined Trowell (1948) emphasizes this point

It follows that if a survey is to have any value for comparison with other such surveys, the worker must carefully describe the signs he is recording and the point at which they are considered by him to be positive and this I now do.

HAIR.

Dry Staring The Zande normally has short tight black curls. The single subject showing this sign had hair in which the curl was almost absent and the hair lay in straggly dry wisps.

Hypochromotrichia. There is a type of Zande whose skin is paler than that of his fellows and whose hair is reddish-brown instead of black. This distinction appears to be hereditary he is born like this and remains so throughout life. Probably less than 1 in 500 is of this type and none was included in this survey

In the earliest stage of the physical sign under discussion a change from the normal black to a reddish-brown colour is observed in the short hair above the temples where the normal hair-line recedes on each side of the brow In some cases, only the tips showed the change in colour in others the whole shaft of the hair In an advanced stage the hair of the whole head is of the reddish-brown colour and wherever it is seen the hair appears drier and with less curl than usual. This change is undoubtedly reversible in contrast to the " red " Zande described previously nd was seen chiefly among children

CONJUNCTIVAE.

Thickened Grades I and II If the eyelids of one showing this sign be parted with the finger and thumb the triangular area of conjunctiva which lies on each side of the cornea and which is normally exposed when the eye is open, is found to be thickened in contrast to the conjunctival surface which is normally covered by the lids. It is usually of a yellower colour with irregular swollen areas, and it is often irregularly pigmented.

Where this thickening and irregularity covered the whole of the orbital conjunctiva, both on the exposed and the normally covered surfaces, it was considered as Grade II It is probable that many or even most of these Grade II cases, would have been classed by other workers as *xerophthalmia* (Thus ADAMSON PLATT *et al* 1945 and PASSMORE, 1947).

Bitot's Spots. Glistening white plaques, adherent to the tops of irregularities on the thickened conjunctiva and probably composed of dead epithelium, form an unmistakeable physical sign.

Circumcorneal Injection.—Only very definite and obvious dilatation of the blood vessels surrounding the cornea would have been considered as a positive sign under this heading and none was seen though a degree of increased vascularity of the conjunctivae as a whole was not uncommon.

Blepharitis Generalized chronic inflammation of the eyelids was not seen.

FOLLICULOSIS

Acne-like lesions seen particularly on the forehead and cheeks, sometimes with visible soft sebaceous accumulations (PLATT, 1945)

DYSSEBACIA

A sign which is definite Whitish-yellow filiform plugs of sebaceous material stand out in plain contrast with the surrounding black skin and are not wiped away by a firm brush with the finger The condition is usually seen on chin and cheeks but may involve the forehead, the skin at the base of the neck and that over the sternum The area involved varied considerably, but where even a small patch of skin showed the change the sign was marked as positive An excellent photograph of the condition was given by PLATT (1945)

ANGULAR STOMATITIS

Loss of elasticity at the angles of the mouth with the appearance of inflamed cracks and soreness

CHEILOSIS

Sore red lips with a thin, glazed, wrinkled epithelium without elasticity

TONGUE

Swollen Marks on the side and front of the tongue due to indentations of the teeth have been described as diagnostic of the swollen tongue It was observed in this series that these indentations may occur when the tongue is clearly not swollen, due perhaps to an abnormally wide tongue in a small mouth Persons considered to be showing this sign were those in whom the tongue appeared thickened as well as indented

Papillae Hypertrophic Fig 20, in " Medical Survey of Newfoundland " (ADAMSON, PLATT, *et al*, 1945) shows the condition to perfection Enlarged papillae stand out a deep red in contrast to the paler surrounding surface In some cases only the tip of the tongue is involved, and these too were considered to be positive

Papillae Atrophic A smooth glistening surface, usually on a thin atrophic tongue

Fissured Cracks of varying depth, usually longitudinal and usually found on a small red tongue In general, such a tongue was not sore

Magenta Coloured A difficult sign Only if the colour was of a deep purplish-red was the sign considered positive, doubtful colours being ignored

CLINICAL ANAEMIA

This was estimated by observation of the tongue and mucous membranes To check the accuracy of such observations, haemoglobin estimations of 12 persons who were considered clinically to be anaemic, were done by both the

Sahli and Tallqvist methods. By the Sahli technique all showed figures of 83 per cent. (11·62 gramme per 100 ml.) or less nine showed figures of 80 per cent. (11 2 gramme per 100 ml.) or less, and five less than 74 per cent. (10·36 gramme Hb per 100 ml.) The Tallqvist method, as has been experienced previously gave results which showed no correlation with either Sahli or clinical estimations.

From these figures it is concluded that those marked as positive under this heading had less than 11 7 gramme haemoglobin per 100 ml. and most considerably less, though some cases of anaemia were probably missed.

GUMS

Swollen. Noted particularly in the interdental papillae

Bleed Easily The upper lip was pressed against the gums with a finger and rubbed from side to side. If bleeding occurred the sign was present though clearly the stimulus was slight.

Gingivitis A definite inflammation f the gum margins.

Carious Teeth. A dental probe was not used and minor degrees must therefore, have been missed.

SKIN

Permanent Gooseflesh Follicular Keratosis

The name of the former sign ccurately describes the appearance. The condition wa found particularly over the hips and above the elbows in women. In the former site it appears to be connected with the constant rubbing of the skin by the baby which is usually carried at the side. The condition is also found commonly just above the knees and about the base of the neck in men.

TROWELL (1948) considers that "permanent gooseflesh" is but an early stage of follicular keratosis, the latter term being kept for those cases where the prominent follicles have become more keratinized and are not only to be seen but to be felt. With this my observations agree, but it seems that whereas "permanent gooseflesh does not always occur only in those parts subject to trauma, follicular keratosis requires exposure and a degree of trauma for its formation. In this series it was seen only on the extensor surface above the elbows, on the thighs, and occasionally above the hip but never at the base of the neck. There was never any doubt as to the presence or absence f permanent gooseflesh but the change to follicular keratosis was indefinite and only advanced changes were considered under the latter heading

Crackled Sk n

Dry Skin G ade I It was exceedingly difficult to decide firstly whether the skin of the legs was drier than normal, and secondly whether the change to crackled skin had occurred. The condition showed a gradual transition from the soft suppleness of normal skin to the dry cracked, desquamating surface of th well-established condition.

If there appeared to be less elasticity than expected in the surface layer of the skin below the knee, and particularly over the tibial surface, dry skin, Grade I, was diagnosed If the change had proceeded to fine reticulation and cracking of the surface with fine desquamation at the edges of the cracks, " crackled skin " was noted as present It appears that in the past some writers have described as crazy-pavement dermatosis these minor changes confined to the skin of the leg below the knee (NICHOLLS, 1940, McKENZIE, 1941) I agree with TROWELL (1941) that the former term should be reserved for the more severe changes where deeply pigmented patches tend to peel disclosing pale underlying areas No case of true crazy-pavement dermatosis was seen in this survey though some cases have been seen in hospital practice

Those with " crackled skin " were automatically marked as having dry skin, Grade I, as well

Dry Skin Grade II This heading included those with dry skin not only on the legs but over the body as well

ULCERS AND ULCER SCARS

The lower part of the legs of the Azande are, not infrequently, marked with pigmented scars of various shapes and sizes Many are the result of septic abrasions, others of developed ulcers In this series only those who had an active tropical ulcer, or who showed scars which had definitely deformed the contour of the skin, thus giving evidence of previous deep ulceration and healing with fibrosis, were noted as positive under the heading The use of these criteria excluded those who had previously suffered from only shallow sores

SYMPTOMS

No attempt was made to assess the prevalence of symptoms which might be attributable to nutritional ill-health " Listlessness, lack of enterprise and interestedness," which PASSMORE (1947) notes as important evidence of early malnutrition, are certainly seen by all those who employ labourers, but how far these characteristics are due to poor nutrition and how far to other factors, such as endemic diseases, it is impossible to say

THE PREVALENCE OF THE SIGNS

Table I shows the numbers in each of the five groups who showed the signs which have been described

Table II gives the figures divided into peasant and wage-earner groups

It will be observed that for some of the signs less than the total number in the group has been considered This is explained by the fact that, in the early part of the survey, only some of the signs were registered, the rest being neglected In such cases, the number in the group in whom the sign was specifically looked for, is indicated by the letter beside the number in the table (A, B, etc)

TABLE I

PREVALENCE OF THE SIGNS (BY AGE AND SEX GROUPS)

	Group I boys.	Group II girls.	Group III men.	Group IV women.	Group V special.	TOTAL No.	
Number in group	185	154	92	122	50	603	100
HAIR, Dry staring	1	0	0	0	0	1	0 17
Hypochromotrichia	21	26	1	3	3	54	9 0
CONJUNCTIVAE, thickened							
Grade I	75*	29A	41B	66C	14D	225E	61 9
II	3*	4A	37B	16C	4D	64E	13 0
Bitot's spots	1	0A	0B	0C	0D	1E	·7
Circumcorneal injection	0*	0A	0B	0C	0D	0E	0
EYES. Blepharitis	0*	0A	0B	0C	0D	0E	0
FACE. Folliculosis	18*	14A	13B	14C	9D	65E	17 8
Dyssebacia	10*	[illegible]A	49B	44C	6D	117E	33 6
Angular stomatitis	1	0	1	0	1	3	5
Cheilosis	1	0	0	0	0	1	17
TONGUE. Swollen		3	12	14	4	35	5
Papillae hypertrophic	136	110	76	64	28	385	64
atrophic	8	3	7	5	0	22	3 7
Tongue fissured	4	5	5	2	0	16	7
magenta coloured	1	0A	1B	0C	0D	2E	33
CLINICAL ANAEMIA	21	18A	15B	34C	7D	95E	4 4
GUMS. Swollen	17*	8A	10B	17C	3D	55E	13 3
Bleed easily	3*	3A	1B	3C	0D	10C	
Gingivitis	21	18	85	45	21	190	[illegible] 5
TEETH. Carious	15	1	19	37	12	85	15
SKIN.							
Permanent goose-flesh	49	50	39	36	18	194	32 3
Follicular keratosis	2	8	4	0	2	16	3 7
Crackled skin	93	64	3	31	15	206	34 7
Dry skin Grade I (legs)	75*	59A	43B	50C	10D	231E	[illegible]
II (body and legs)	11	1A	2B	11C	0D	25E	[illegible]
ULCERS AND ULCER SCARS	4	47	19	36	14	127	[illegible]

* Total examined	110	C Total examined	[illegible]
A	75	D	23
B	69	E	365

TABLE II

PREVALENCE OF THE SIGNS (BY SOCIAL GROUPS)

	Not resettled	1st year resettled	2nd year resettled	Wage-earners Yambio police	Mission school
Number in group	100	151	176	68	108
HAIR Dry, staring	0	0	0	0	1
Hypochromotrichia	19	21	5	0	9
CONJUNCTIVAE, Thickened					
Grade I	\	9H	101	52	64
" II	\	3H	33	14	4
Bitot's spots	\	0H	0	0	1
Circumcorneal injection	\	0H	0	0	0
EYES Blepharitis	\	0H	0	0	0
FACE. Folliculosis	\	1H	40	0	24
Dyssebacia	\	5H	39	55	18
Angular stomatitis	0	1	1	0	1
Cheilosis	1	1	0	0	0
TONGUE. Swollen	5	16	8	5	1
Papillae hypertrophic	81	96	95	27	66
" atrophic	2	3	6	5	6
Tongue fissured	0	0	7	2	7
" magenta coloured	\	0H	1	0	1
CLINICAL ANAEMIA	\	5H	37	22	25
GUMS Swollen	\	0H	43	1	11
Bleed easily	\	0H	6	1	3
Gingivitis	23	43	58	22	13
TEETH Carious	7	42	31	0	6
SKIN Permanent gooseflesh	26	37	51	28	52
Follicular keratosis	0	5	5	6	6
Crackled skin"	24	14	96	4	70
Dry skin grade I (legs)	\	12H	122	40	77
" " II (body and legs)	\	0H	25	0	0
ULCERS AND ULCER SCARS	37	17	22	10	32

\ = Not registered H = Total examined 13

THE DIET

Mrs G. M. CULWICK has supplied the following preliminary note and Tables III and IV showing the dietary intake

The staple food is cassava, with eleusine and sweet potatoes as secondary staples. Groundnuts are by far the most important accessory food and, nutritionally speaking, the salvation of the diet in the extent to which they make good the shortcomings of cassava. Owing to the lack of livestock, animal products play a relatively small part, but termites make an appreciable contribution during a short season. Mangoes are eaten in very large numbers during the early rains, they and the leafy vegetables and sweet potatoes are, in turn, the main sources of vitamins A and C through the seasons

Evaluation of the quantitative dietary data gives the results shown below. The figures refer to food only and do not include beer, which was a variable item. Estimates of calorie and protein requirements are given, the former based on body-size, age, activities, etc., on the lines laid down in the draft report of Platt's nutrition survey in Nyasaland (1938–39), and the latter on the rates for weight and age given in the 1948 edition of the National Research Council Recommended Allowances.

PLATT (1946) gives a table of values for nutrients recommended as an immediate objective for feeding populations in the West Indies. It is based, with modifications, on the recommendations of various official bodies, and is

TABLE III.

PEASANT GROUP NUTRIENT INTAKE PER HEAD PER [illegible]

Season	Early rains.	Late rains.					Dry season.		
Group	A.	A.	B.	C.	[illegible]	D.	A.	C.	D
Number of homes ...	37	47	33	70	4	[illegible]3	40	25	23
Person-days	360	349·5	322	777	332	43	300	2[illegible]1	[illegible]20
Calories	1,828	2,275	2,030	3,073	2,775	3,039	[illegible],400	[illegible],450	3,573
Protein, g.	33	41	40	43	60	71	36	32	79
Fat, g.	21	30	34	22	57	67	49	44	71
Carbohydrate, g.	330	437	417	395	503	538	447	404	384
Calcium, g.	0·7	1·0	0·7	0·8	0·8	1·6	1·2	1·3	1·7
Iron, mg. ...	18	21	16	30	1[illegible]	29	1[illegible]	34	47
Vitamin A, I.U.	9,000	7,300	6,700	4,900	3,700	3,900	3,700	1,200	4,100
Aneurin, mg.	0·9	1·2	1·3	1·3	1·9	2·2	1·4	1·1	1·4
Riboflavin, mg.	0·5	0·5	0·5	0·4	0·6	0·6	0·8	0·5	0·8
Nicotinic acid, mg.	14	19	19	18	17	17	23	14	19
Ascorbic acid ( ), mg.	85	70	28	12	29	1	31	15	39
raw value mg.	164	109	117	79	101	93	94	31	71
Protein/carbohydrate,	9·7	9·4	9·8	10·8	11·9	13·2	12·6	12·9	[illegible]
Estimate of calorie requirements (b)	2,400	2,300	—	3,150	[illegible]	[illegible],150	2,500	2,2[illegible]	2,300
Estimate of protein requirements, g. ( )	58	63	—	51	57	66	57	51	58

( ) Estimate of actual intake

(b) Based on body-size, age, activities, etc.

( ) National Research Council rates for weight and age

A. Not yet included in re-settlement scheme

B

C 1st year settlement.

D 2nd

given on a per head basis for a population of mixed age and sex groups It will be useful as a basis for comparison and evaluation of the Zande diet and is, therefore, given as Table V For comments on the individual figures, PLATT's paper should be consulted It should be noted that an estimate of calorie and protein requirements based on the data from the tribe itself is given at the foot of Tables III and IV

A comparison of the dietary intake of the Azande with these figures for estimated requirements shows the most outstanding deficiency to be that of riboflavin which never exceeds one-third of the estimate The intake of protein is on the low side during the rains, whilst that of vitamin A is low in the dry season The intake of other essential nutrients appears on the whole to be reasonable, with iron and ascorbic acid slightly on the low side in some groups and more especially among the wage-earners

TABLE IV

WAGE EARNERS NUTRIENT INTAKE PER HEAD PER DAY

Season	Early rains	Late rains	Dry season		
Group	A	A	B	A	C
Number of homes	48	47	39	32	—
Person-days	647	583 5	248	388	40
Calories	2 075	2,125	2,775	2,375	2,175
Protein, g	55	35	55	50	45
Fat, g	45	41	58	46	35
Carbohydrate, g	338	403	502	437	425
Calcium, g	1 0	0 7	1 1	1 0	0 9
Iron, mg	19	15	24	18	16
Vitamin A, I U	5,700	4,900	1,300	2 200	2,450
Aneurin, mg	1 1	0 9	1 5	1 2	0 8
Riboflavin, mg	0 6	0 4	0 5	0 5	0 4
Nicotinic acid, mg	19	18	24	21	17
Ascorbic acid (a), mg	138	19	22	17	11
,, raw, value, mg	157	93	55	61	51
Protein/carbohydrate, per cent.	16 3	8 7	11 0	11 5	10 6
Estimate of calorie requirements (b)	—	—	2,500	—	—
protein requirements, g (c)	—	—	55	—	—

(a) Estimate of actual intake

(b) Based on body-size, age, activities, etc

(c) National Research Council rates for weight and age

A Hospital and general station staff, Li Yubu, Family units

B Police, Yambio Family units

C Nutrition survey staff Li Yubu No families

TABLE V

VALUES FOR NUTRIENTS RECOMMENDED AS AN OBJECTIVE FOR FEEDING POPULATIONS IN THE WEST INDIES (PLATT 1946).

Calories	2,800	Vitamin A (as β carotene)	5,000 I.U.
Protein	60 g.	Aneurin (vitamin B_1)	1·8 mg.
Calcium	0·8 g.	Riboflavin	1·8 mg.
Iron ...	20 mg.	Nicotinic acid	12 mg.
		Ascorbic acid (vitamin C)	30 mg.

THE SIGNIFICANCE OF THE SIGNS.

Before going on to the interpretation of the physical signs in terms of possible nutritional deficiencies, it is as well to review shortly the endemic diseases of the area, their prevalence and the possible roles they may play in producing the appearances described.

Filariasis due to *Loa loa* and to *Acanthocheilonema perstans* is exceedingly common. Onchocerciasis is relatively rare except in certain localities where it also is common but these were not chosen for the survey. The former two parasites are thought by some to cause changes in the skin similar if not identical with those described. It is possible that they may have played a part in the production of signs noted in this survey but I personally am doubtful whether their role is an important one. *Onchocerca volvulus* certainly causes skin changes. In areas where this parasite is prevalent, dryness and thickening of the skin are common though the parasite may be found in skin which shows no such changes (KIRK, 1947).

Bilharziasis due to *Schistosoma mansoni* and ancylostomiasis due to *Ancylostoma duodenale* are found in more than 25 per cent. of persons and undoubtedly play a part in the causation of anaemia. On the whole the Azande are resistant to these parasites and symptoms are rarely as severe as are found in other tribes.

Malaria, due mainly to *Plasmodium falciparum*, is hyperendemic and causes noticeable anaemia, particularly in infants.

Syphilis is widespread though yaws is exceedingly uncommon.

Leprosy occurs in just over 5 per cent. of the inhabitants. It may give rise to areas of skin showing typical permanent gooseflesh (see the photograph B in the paper by WOODMAN 1947). These occur in well defined patches, usually small and often over the back and are due to the activity of the disease itself. In some advanced cases atrophy of the skin over the entire body occurs with thinning dryness and loss of elasticity; in others, crazy pavement dermatosis may be observed, but these changes are no doubt due to super-added nutritional defects.

Such a high incidence of leprosy may in itself be an indication of nutritional ill health in a community.

With some appreciation of the prevalence of disease, we can now pass to a consideration of the signs and their possible causes in terms of nutritional poverty

Hypochromotrichia TROWELL and MUKAZI (1945) note a failure to gain weight, some softness and brownness of the hair and pallor of the facial skin as the earliest manifestations of " malignant malnutrition," the cause of which has recently been under discussion (TROWELL, 1949) Pallor of the facial skin was not specifically looked for in this survey but does occur in conjunction with the hair changes PLATT (1946) classes the sign among those usually regarded as due to vitamin B_2 deficiency or to lack of certain amino-acids TROWELL (1948) observes that signs in the hair cannot be ascribed to a deficiency of any individual vitamin though they " sometimes slowly improve if a diet rich in calories, in animal protein, in liver and in the B_2 complex is given " The work of HUGHES (1946) suggests that a deficiency of pantothenic acid may be a cause

The condition occurs mainly in the younger age-groups and is undoubtedly reversible Evidence from this survey lends support to the view that a deficiency of certain parts of the B_2 complex is the cause

Conjunctival Changes TROWELL (1948) and ADAMSON, PLATT *et al* (1948) consider these changes as probably due to avitaminosis A, though PLATT (1946) states

> " I am impressed with the association found between the incidence of vitamin B_2 (and/or protein) deficiency, such as these skin changes, and conjunctival changes grouped under " Excess Tissue, grades 1, 2 and 3 "

In this series also, the association between skin and conjunctival changes is well marked

The report of the Medical Research Council (1949) noted that the " occurrence (of these conjunctival changes) did not vary with the vitamin A intake " in their experiments on deprivations lasting up to 25 months

One cannot help feeling that in some degree the heat and glare of a tropical climate combined, in the dry season, with irritating dust, must account for changes in the exposed portions of the conjunctivae This is borne out by the observation that the changes are most frequently observed in the adult groups and are least common in young girls, but this cannot be the whole truth for adults can be found who do not show the change

Skin Changes Folliculosis, dyssebacia, crackled skin, " permanent gooseflesh ", dry skin, Grades I and II

PLATT (1946) states that it is usual to regard these changes (among others) as features of vitamin B_2 deficiency, but he adds

> "There is, however, reason to think that some confusion exists in experimental work between the effects of B_2 vitamins and those of certain amino-acids, and it may be that *some* of the effects ascribed to shortage of B_2 vitamins will ultimately be found to be due to a shortage of certain essential amino-acids "

He goes on to note his impression that these changes are associated with conjunctival changes in the passage quoted above

In his paper of 1945 he draws attention to the work of SMITH, SMITH and CALLAWAY (1941), who produced evidence that dysebacia does not respond to treatment with known members of the B_2 complex, but is cured by administration of autoclaved yeast or liver extract.

Follicular keratosis PLATT (1946) considers separately from the above skin changes, and observes that with Bitot's spots the sign is generally regarded as evidence of vitamin A deficiency He noted in 1945 however that CRANDON, LUND and DILL (1940) had shown " quite conclusively that a follicular keratosis can be produced in man on a vitamin C deficient diet containing adequate amounts of vitamin A.

TROWELL (1948) considers follicular keratosis t be the ultimate stage of a change which starts as permanent gooseflesh " and groups them both under the heading of those signs commonly ascribed to a deficiency of vitamin A.

A most significant work, however was that undertaken by the Medical Research Council (1949) which led to the conclusion that the tendency to develop Keratinized Hair Follicles was not specifically related to the state of Vitamin A nutrition," though this conclusion cannot necessarily be applied to deficiencies lasting over many years and in tropical climates.

In this survey little assistance can be given to the elucidation of the matter for both the B_2 complex and vitamin A were deficient in the diet during the dry season.

I am of the opinion that dry skin, Grade I and the lesser degrees of crackled skin are, in a great many cases, merely the result of the climate and the habits of the native. Hot, dry weather sitting about or working in the dust all day and, in the evenings, warming the legs beside a fire, must all be conducive to drying of the skin, and absence of washing allows the dry surface layers to remain undisturbed. After long dry treks I have observed on my own legs a drying of the skin which I would have classed as a positive sign under the heading and on some occasions I have seen minor degrees of crackled skin over my shins, though the change has not remained for long

I would agree that severe degrees of the latter condition are a manifestation of a nutritional disorder but the dividing line between the normal and the pathological is so indistinct and depends so much on the observer that th sign is virtually useless as an indication of nutritional ill health n comparati nutritional surveys. The signs of dysebacia permanent gooseflesh and folliculosis, are much more satisfactory in that the deviations from the normal appearance are distinct.

SIGNS IN THE TONGUE

Hypertrophied papillae were seen in over 60 per cent. of persons examined, whereas the other changes were uncommon. ADAMSON PLATT *et al* (1945), who reproduce a photograph which show exactly the condition found in this series, state

" A less severe chronic deficiency of Niacin causes hypertrophy of papillae of the tongue, followed by multiple fissuring and papillary atrophy "

If all the cases seen were indeed due to niacin deficiency, it is surprising that later stages of the change were not seen more frequently Moreover, the dietary investigation does not indicate any shortage of this vitamin in comparison with recognized standards

ANAEMIA

It is most noticeable that severe anaemias which, in many other tribes, result from bilharzia and hookworm infections, are rare among the Azande Nevertheless, mild and moderate degrees of anaemia are common and must result from an unsatisfactory balance between blood loss and destruction and the intake or absorption of factors necessary for its regeneration The type of anaemia was not studied in this investigation but hospital experience points to the microcytic, hypochromic variety as being the most common The incidence was highest in adult women and in those pregnant or suckling babies The dietary records show a comparatively good intake of iron

CHANGES IN THE GUMS

Swollen gums were seen in 15 per cent and gingivitis in 26 per cent Bleeding gums were noted in less than 2 per cent , but the trauma applied was minimal

ADAMSON, PLATT *et al* (1945) consider that these changes can be caused by a low intake of ascorbic acid They report that in a recent study in Canada " there is evidence to show that many of the acute and subacute gingival signs are the result of an increased liability to infection " They could be caused to disappear by local treatment, but with a low intake of ascorbic acid recurrences were more frequent than in those with an adequate intake TROWELL (1948) considers the evidence to be conflicting

The ascorbic acid intake was, on the whole, slightly lower than the estimated requirement

TROPICAL ULCERS AND SCARS

The cause of this common condition has never been satisfactorily explained Though many workers have published papers giving evidence to incriminate single substances, their claims have not been substantiated It is clear that there are a number of aetiological factors at work, and it is safe to assume that one or more of them is nutritional A high incidence of tropical ulcer may provide evidence of nutritional ill-health in a community, but further than this we cannot, at present, go

SUMMARY AND CONCLUSIONS

This clinical survey has shown that signs commonly ascribed to nutritional ill-health are exceedingly prevalent among the Azande

The preliminary dietary findings reveal a serious shortage of riboflavin which never exceeds one-third of the estimated requirements. It seems possible that this deficiency may be responsible for the very high incidence of conjunctival changes and the skin changes described as "permanent gooseflesh," "crackled skin" and dysaebacia, though a lack of certain amino-acids found in conjunction with this vitamin cannot be excluded. The picture is further complicated by the observation that vitamin A is short of estimated requirements during the dry season.

In this paper a table of estimated dietary requirements given by PLATT (1946) has been used as a yardstick with which to assess the value of the Zande diet. It is, however, possible and indeed probable that an ideal diet for a Zande should contain the main nutrients in quantities very different from these estimates. It is suggested that different tribes in Africa and in other parts of the world will react differently to any given diet just as they show different reactions to the same disease and to the same medicine (See BRYANT and FAIRMAN 1940 for some striking examples of this difference in the Azande and Dinka tribes.) A "Standard" of dietary requirements for African tribes, when it comes, will need to be stated in very broad and variable terms, and the knowledge on which it will be based will be acquired by survey of different groups in which both the dietary intake and the clinical signs of ill health are evaluated and correlated, and it will be important for purposes of comparison that the latter be carefully described. Moreover due cognizance must be taken of the parasites which the African supports, and any estimate of his "Standard" needs when this is to be usefully applied to primitive areas, would do well to include provision for them.

REFERENCES

ADAMSON, J D *et al* (1945) *Canad med. Ass. J* 52, 227
BRYANT J & FAIRMAN H. D (1940) *Trans R Soc trop Med Hyg* 34 117
CULWICK, G M (1949) *Preliminary Note on Dietary Findings* Private communication.
HUGHES, W (1946) *Brit. med J* 2 85
KIRK, R. (1947). *Ann. trop Med Parasit* 41 357
MEDICAL RESEARCH COUNCIL. (1949) *Vitamin A Requirements of Human Adults* Special Report Series, No. 264 London H.M.S.O
MCKENZIE, A. (1941). *Trans R. Soc trop Med. Hyg* 35 47
NICHOLLS, L. (1940). *Ibid* 34 291
PASSMORE, R. (1947). *Ibid* 41 189
PLATT B. S. (1945) *Brit med Bull* 3 179
—— (1946). *Nutrition in the British West Indies* Colonial No. 195. London H.M.S.O
—— (1947). *Trans R. Soc trop Med. Hyg* 40 379
TROWELL, H. C. (1948). *E Af med J.*, 25 311
—— (1949). *Trans. R. Soc trop Med. Hyg*, 42 417
—— & MUWAZI, E. M. K. (1945). *Ibid* 39 229
WOODMAN, H. M. (1947) *E Afr med J* 24 315

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STUDIES IN ONCHOCERCIASIS *

(A REVIEW OF 100 CASES FROM ENUGU DISTRICT OF EASTERN NIGERIA)

BY

CHUKUEDU NWOKOLO

(From the Out-patients Department, General Hospital, Enugu, Nigeria)

GOLDMAN (1944) consıders the clınıcal syndrome of Amerıcan onchocercıasıs to consist of onchocercomata (the nodules), onchodermatıtıs (the rash), and onchophthalmıa (ocular manıfestatıons) Varıous wrıters (ROBLES, GOLDMAN and ORTIZ, MANSON-BAHR, GOSPILL) also mentıon epılepsy as a possıble complıcatıon of the dısease, and ın theır account, GOLDMAN and ORTIZ (1946) recorded that 10 per cent of theır Mexıcan serıes were epıleptıcs Workıng ın East Afrıca, GABATHULER and GABATHULER (1947) ıncrımınated onchocercıasıs ın the aetıology of muscular abscesses The same authors also descrıbed a case assocıated wıth habıtual abortıon From French West Afrıca, DEJOU (1939) descrıbed acute arthrıtıs ın cases of onchocercıasıs and ısolated mıcrofilarıa of *Onchocerca* from the affected joınts

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That some form of dermatitis is associated with onchocerciasis is well known to all students of tropical medicine. The studies of GOLDMAN and ORTIZ (1946) and many others show that American onchodermatitis consists of the following types The *erysipela de la costa* or *de la morado*, found chiefly in cases with onchocercoma of the scalp and characterized by a generalized or localized erysipeloid infection of the face beginning with constitutional disturbance pain, redness and swelling of the face. The chronic form shows ichthyosis of the skin and is said to resemble myxoedema. Localized pigmentation of parts of the face is common. Then there is the other type of dermatitis characterized by localized oedema and elephantiasis. There is also the lichenoid form in which the skin is dry thick and rough. Another type consists of acute subacute and chronic eczematoid reactions. A skin affection not strictly classed as onchodermatitis is the reaction to the bite by a simulid which LOWENTHAL (1943) considers has not the same cause as the papular onchodermatitis. It consists of pruritis, central petechial haemorrhage and oedema and lasts only for a few days. Any of these pruriginous forms could be complicated by pyoderma due to infection of scratch marks.

Not all types of onchodermatitis satisfy the above description, and this account attempts to present the typical findings in a series of cases of onchodermatitis in Enugu, West Africa.

ONCHODERMATITIS IN ENUGU NIGERIA

In certain parts of Nigeria, including parts of the provinces east of the Niger onchocerciasis is endemic, and it is a common experience to see an African suffering from a skin condition coming to the hospital, and asking to be relieved of certain tumours believed to have caused the dermatoses. As often as not, the incriminated nodule is only the chronically enlarged regional lymph gland draining the area of secondary infection following the skin disease but without doubt, this widespread belief originated from the often dramatic disappearance of onchodermatitis following extirpation of the associated onchocercoma. The operation has probably been practised in these parts for many centuries, and highly skilled specialists in the art still abound in endemic areas. Such areas present a fertile field for the study of onchocerciasis.

Observed Clinical Signs and Symptoms of Onchodermatitis

The earliest symptom of an onchodermatitis is pruritus. When the skin is scratched a diffuse papulomacule of variable size appears. In severe attacks characterized by very severe pruritus, the initial papulomacule is relatively large *i.e.* ½ inch by ½ inch, and adjacent papulomacules show a tendency to coalesce. In milder cases, the lesions may be ¼ inch in diameter or even smaller and there is a shift towards discreteness. The edge of the papulomacule in the earliest stage fades imperceptibly into the normal surrounding skin. The top is flat. As the lesion grows older *e.g.*, 1 week to 2 weeks its edge becomes

more definite and the initial papulomacule becomes a definite papule with smaller dimensions. The next stage varies in different people and different areas on the same patient. The papule may become infected by staphylococci or streptococci and pustulate. The pustule soon liberates its contents, and a scar is formed. In more resistant patients, the papule may persist for a long time and may ultimately disappear without being secondarily infected. The top of the papule may also be scratched off, exposing the raw base. This either becomes infected or it heals. In either case, the end result is the same—a small scar about $\frac{1}{8}$ inch in diameter formed over the site of the former papule. The scar soon epithelializes, but the epithelium is at first devoid of pigment, and the pigmentation is characteristically and exceptionally slow in coming. The final result in severe attacks is clearly shown in the photograph of Case 28—the skin presenting a mottled mosaic of black and white, the white representing the hypopigmented patches, which are often seen. By the time this stage is reached, the skin is covered with nail marks, and considerable lichenification occurs and is more marked over the areas of the body most accessible to the nails. It would be incorrect to label this lichenification as part of the syndrome of onchodermatitis because it occurs to a greater or lesser extent in all other pruritic dermatoses, including chronic urticaria and even scabies.

The true characteristic onchodermatitis consists of the papulomacules and papules described above. These represent the types most frequently encountered in endemic areas. Certain variants of the above also exist. Sometimes the lesions are finer than indicated above and more closely set but otherwise show the same general characteristics as regards distribution and pruritus. In other cases, the papules or papulomacules persist almost indefinitely and the lichenification progressively masks these elementary lesions.

A certain amount of oedema of the skin usually accompanies the early lesions of onchodermatitis. This is more evident when the lesion starts in any of the limbs (photographs, Cases 46 and 83) when the resulting asymmetry is obvious. The oedema does not persist and lessens as the lesion grows older. In cases of up to 1 year's duration, it is usually absent or very mild. In keeping with that observed in other filarial conditions, the oedema due to onchocerciasis does not pit on pressure. The elephantiasis never attains the size encountered in *Wuchereria bancrofti* infections, and usually results only in slight but obvious asymmetry.

Distribution of Lesions in Onchodermatitis

One of the most striking characteristics of onchodermatitis seen in Enugu area is their distribution. For descriptive purposes, it might be convenient to divide them into two types (*a*) The regional type, (*b*) the generalized type. It must be emphasized here, however, that there is no fundamental difference between the two types. In fact, in most cases, the regional type represents an early stage of the generalized type.

(*a*) *The Regional Type* Usually but not invariably one of the limbs is attacked alone. In the upper limb it frequently starts on the external aspect of the arm and then spreads downwards, but the arm is in the end more severely attacked than the forearm. When the lower limb is affected, it starts on the upper thigh and the region above the knee is more severely affected than the portion below it. The general tendency is for the skin condition later to spread centripetally and in the end the whole body is affected. This may take 6 months or may be delayed for 2 or more years. The picture of a fully developed regional onchodermatitis is very striking and characteristic. The affected limb is swollen and shows the characteristic maculopapules and papules while the rest of the body remains remarkably free. (Photographs, Cases 68, 83 and 46.) In some cases, this regional type starts on the trunk and one-half of the body may be affected while the other half remains free (photograph 45).

Many cases of regional onchodermatitis are associated with regional onchocercomata e g Case 90 had onchodermatitis of left leg and an onchodercoma over the corresponding greater trochanter Case 89 similarly except that the right side was affected. Case 75 (see photograph) had regional onchodermatitis affecting the left side of the chest wall and immediately surrounded an onchocercoma. It may well be that all cases of regional onchodermatitis are associated with regional onchocercomata but that in some these are not demonstrable either because of their small size or deep location or through being mixed up with the regional lymph glands which are usually enlarged.

(*b*) *Generalised Onchodermatitis* In generalized onchodermatitis, on the other hand, all four limbs and the trunk are affected, but the emphasis is usually on the proximal parts of the limbs, the buttocks, the lower abdomen, and the upper part of the back. The face invariably escapes, while the lesions are minimal over the upper part of the front of the chest. Onchodermatitis may disappear of its own accord. Cases are on record where it appeared stayed on for a year then disappeared only to reappear later The factors which govern its appearance and disappearance are unknown.

Out of a total of 59 cases of onchodermatitis, 20 cases were of the regional type and 21 cases of the total were associated with onchocercomata.

Pruritus in Onchodermatitis

The pruritus of onchodermatitis is a very serious problem in affected patients. In one patient of this series under review (Case 18) who committed suicide, investigation revealed that the pruritus played no small part in producing the abnormal psychic background which led to suicide. It is usually refractory to the usual anti pruritic remedies, and seems to be aggravated by humidity and perspiration.

Many of the patients complained that the itching was worse in the following conditions (*a*) When it rained or threatened to rain (*b*) when they perspired (*c*) when they removed their clothes (*d*) at night and (*e*) after a bath.

CASE 28.—Advanced onchodermatitis 18 months' duration showing extensive scarring and lichenification. A scar is visible over the left side of the root of the neck. An ? onchocercoma was removed from there a year ago

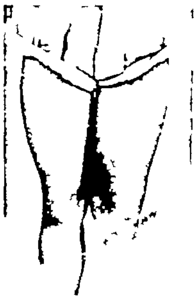
CASE 45.—Regional onchodermatitis which started from left arm and in spreading towards centre has invaded half of trunk. Duration—3 months. No onchocercomata

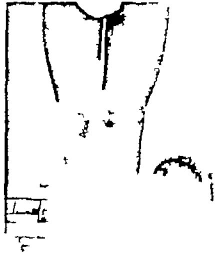
CASE 68.—Regional onchodermatitis right upper limb associated with onchocercomata of right wrist and right anterior superior iliac spine. Duration of onchodermatitis—3 weeks. Oedema present

Same Case 68 few weeks later after removal of onchocercomata. Notice that the lesions over the right fore-arm have entirely disappeared and much of associated oedema now subsided

CASE 46.—Regional onchodermatitis duration 4 months associated with slight oedema but no onchocercomata.

CASE 5.—Onchodermatitis localized around onchocercoma of rib. When patient w... seen one week after remo... al of onchocercoma, most of the lesions had cleared. Unfortunately contact was lost with this patient later and ... second photograph could not therefore be taken.





CASE 83.—Regional ... of left leg ... th oedema ... ms of left knee. Durat... Microfilaria of Onchocerc... from centrifuged deposit of

Some of the factors enumerated above seem to bring about an alteration of the thermal condition of the cuticle Whether or not this is related to the thermotactic tendency of microfilariae (MANSON-BAHR) remains to be proved

Diagnosis of Onchodermatitis

The condition described above, the various stages of which are represented in the accompanying photographs, was recognized as onchodermatitis on the following grounds

(1) Condition when associated with onchocercomata disappeared or improved considerably after extirpation of the onchocercomata (2) They occurred frequently, and constantly in endemic areas (3) When not associated with onchocercomata, showed distribution and qualities in every way identical with those associated with onchocercomata Confirmation was obtained in one such case of regional onchodermatitis of 4 weeks' duration without onchocercomata, but associated with a synovitis of the left knee Active microfilariae of *Onchocerca* were recognized microscopically from the centrifuged deposit of the synovial fluid aspirated from the affected knee joint (photograph, Case 83) (4) In most cases where onchocercomata were also diagnosed they were removed and incised to demonstrate adult filariae All doubtful cases were subjected to microscopy and useful confirmation obtained thereby

Percentage of Onchocerca *Infections showing Dermatitis*

The actual percentage of cases of onchocerciasis showing dermatitis is probably 3 to 7 Hospital figures show a much higher percentage because the dermatitis gives rise to greater discomfort as a result of pruritis For instance, out of the first 100 cases collected in Enugu Hospital, Eastern Nigeria, the figures were as follows Onchodermatitis 59 Of this number, 38 had no demonstrable onchocercomata

About 8 miles from Enugu, around the aerodrome, there is a focus of high onchocercal endemicity Out of 47 labourers working on the aerodrome at the time of examination recently, 17 complained of onchocercomata (verified) of which two showed definite onchodermatitis This gives a figure of about 4 per cent of the total Most of the aerodrome labourers had worked there for intervals varying from 2 to 10 years On the average, those whose tumours developed after arrival in the aerodrome noticed the tumours after working for about 4 years It is not known why the remaining two-thirds remained free of signs of clinical onchocerciasis even after some of them had been exposed to infected simulia in some cases for periods up to 10 years

Blood Picture in Cases of Onchodermatitis

The differential leucocyte count showed Eosinophils, 30 to 40 per cent , polymorphonuclears, 30 per cent , and lymphocytes, 30 to 40 per cent No

constant alteration in the total leucocyte count was found. This is in agreement with the findings of other workers.

Other Signs of Onchocerciasis.

As has been mentioned before onchocerciasis has been associated in various parts of the world with onchophthalmia, multiple myositis, and arthritis.

Onchophthalmia. This was described in detail by STRONG *et al* (1934) but had been recognized before then by earlier workers, including PACHECO LUNA (1918). It is said to give rise to conjunctivitis with photophobia, iritis, keratitis punctata, choroidoretinitis and ultimately blindness. Microfilariae of *Onchocerca* are frequently recognized with the corneal microscope slit lamp and other special instruments. They are commonest in cases with onchocercomata of the scalp.

Of the 100 cases under review there were *seven* with onchocercomata of the scalp Of this number two patients complained of swelling of the face which had arisen since the tumours appeared. All denied having had any visual disturbances although one complained of occasional "redness of the eyes." No objective signs were observed on the simple examination of the eyes. It is possible that a more diligent search will reveal cases of genuine onchophthalmia, but they must be quite rare. This agrees with the findings in certain other parts of West Africa. In fact, the case of onchophthalmia described by SCOTT (1944) is believed to be one of the earliest cases recorded in the Gambia. On the other hand it contrasts sharply with the findings of workers in the Congo, Mexico, and Guatemala, where the problem of onchocerciasis centres on onchophthalmia.

Multiple Myositis Multiple myositis was reported by GABATHULER and GABATHULER (1947) in East Africa to be associated with onchocerciasis, and they claimed to have detected microfilariae in the pus. No microfilaria was found in the pus of a few cases of myositis examined in Enugu Hospital but there is no doubt that further investigation is necessary on this subject.

Arthritis Synovitis and Arthralgia DEJOU (quoted by MANSON BAHR) demonstrated microfilariae of *Onchocerca* from the knee joints of cases of acute arthritis in French West Africa. One case (photograph, Case 83) already mentioned in the Enugu Hospital series under review had synovitis of the knee Microfilariae of *Onchocerca* were demonstrated from the centrifuged deposit of the synovial fluid. The patient also had a regional onchodermatitis over the affected lower limb, but no onchocercoma. Another (photograph, Case 89) who gave a 2 years history of regional onchodermatitis of the right lower limb also gave a history of a previous attack of synovitis of the knee starting about the same time as the dermatitis but which had subsided at the time of examination. He had a small onchocercoma of the greater trochanter on the same side which was 2 years old.

From these cases it seems reasonable to infer that minor arthralgic conditions, and hitherto empirically treated cases of synovitis, might be manifestations of onchocerciasis It might be added here that the author has been struck by the high incidence of vague joint conditions in areas where onchocerciasis is endemic

Onchocercomata

The tumours in African onchocerciasis seem to have a predilection for the trunk while the scalp which is so commonly affected in the South American type is not so frequently attacked In this series, 89 nodules were collected, and they showed the following distributions Iliac crest 22, ribs 17, trochanters 13, spine, especially over sacro-coccygeal region, 11, knee, 10, scalp, 7, scapula, 3, sacro-iliac joint, 2, root of neck, 2, ischial tuberosity, 1, wrist, 1

In the South American series of GOLDMAN and ORTIZ (1946), 40 per cent of the tumours were situated on the scalp STRONG showed that, morphologically, the African onchocerca is identical with the South American type, and suggested that some local trauma such as is caused by the use of leather belts, or the carriage of loads on the head, might be responsible for the high incidence of scalp onchocercomata In making this suggestion, STRONG was probably ignorant of the habits of the West African peoples who also carry loads on the head on a very large scale

The variety of onchocerciasis described here differs from its American variant in its more specific dermatitis, the non-exclusive but definite predilection for the trunk of its onchocercomata, the rarity of onchophthalmia, and perhaps its arthropathic tendencies These differences probably depend on modifications based on geographical or climatic factors In Guatemala, the foci of onchocerciasis were about 1,000 to 3,000 feet above sea level The investigations reported above in Nigeria were conducted at a height of about 630 feet above sea level

Treatment

Most patients with tumours do not complain of pain, hence dermatitis is the chief symptom which requires treatment It has already been pointed out that in endemic areas, African 'doctors,' so-called herbalist-surgeons excise the onchocercomata, where they are demonstrably associated with onchodermatitis This type of treatment was adopted where possible

About 50 per cent of the patients were completely relieved of their dermatitis within 3 weeks of the removal of onchocercomata, 40 per cent were partially relieved in the same period The rest or 10 per cent, showed no observable changes Great difficulty was encountered in following up cases and these figures are at best approximate The poorer results were attributed to the presence of subclinical onchocercomata

Intramuscular injections of pentamidine isethionate were tried on some cases of onchodermatitis showing no onchocercomata Results obtained from such cases were inconclusive Other drugs were not available and the new drug, banocide was not tried

Discussion

Most of the detailed studies on the subject matter of onchodermatitis have been limited to the American variety of onchocerciasis, and an obvious gap exists in medical literature concerning the description of the various types of onchodermatitis.

The African variety or varieties of onchodermatitis have not received the attention they deserve. Southern Nigeria, West Africa in particular forms a fertile soil for any such studies. The studies carried out here have shown that the distribution of onchodermatitis is different from the distribution observed in South America. While other parts of the body are affected, there is a definite predilection for the trunk. No onchophthalmia was discovered in 100 cases reviewed here

The precise aetiology of onchodermatitis is as yet poorly understood. LAIGRET (quoted by FAIRLEY 1946) believes that onchodermatitis is caused by the presence of microfilariae in the skin. This seems very reasonable but it is also known that the greater percentage of cases suffering from proved onchocerciasis do not present any skin lesions. Microfilariae are also frequently isolated from the skins of patients not even clinically suspected of having onchocerciasis. There must, therefore, be some other factors than the mere presence of microfilariae. This is supported by the histological picture observed by GOLDMAN and ORTIZ (1946), and by MANSON BAHR, who pointed out that microfilariae live free in the connective tissues and do not excite any cellular reaction while alive. Perivascular cuffing and oedema are often observed in cases of onchodermatitis, but these bear no relationship either to the number or position of microfilariae. It is quite possible that in some special cases, the skin is sensitized to the onchocerca as suggested by LOWENTHAL (1943).

Another possibility is that certain species of the adult filariae or microfilaria are dermotropic. GABATHULER and GABATHULER (1947) found urea in onchocerca cysts and concluded that urea might be a metabolite of *Onchocerca volvulus*. It may yet be that some other as yet unknown metabolite of *O. volvulus* may be responsible for the pruritus and skin lesions seen in onchodermatitis.

The disease in Nigeria is commoner among the poorer sections of the community. This is possibly due to the scarcity of clothing predisposing to effective simulium bite. While some authors have blamed avitaminosis and associated diseases as the reason for the onchocerciasis among the poorer classes, our studies here show that these are not as important as is the scantiness of the clothing worn by the patients under review

Summary

A review is made of the various manifestations of onchocerciasis encountered in different parts of the world. A detailed description is given of the types of onchodermatitis encountered around the Enugu District, Eastern Nigeria, and attention is directed t certain special features of these especially

their distribution It is estimated that about 3 to 7 per cent of cases of onchocerciasis show onchodermatitis The precise aetiology of onchodermatitis is unknown, and it is suggested that it may be an allergic phenomenon or depend on the presence of some irritant metabolite of the parasite In parallel to the behaviour of viruses, a suggestion of a possible dermotropic species of *Onchocerca* is also made Evidence from a small number of controlled cases shows that about one-third show clinical evidence of infection in areas of proved high endemicity Such high figures are only attained in special areas In the analysis of onchophthalmia, it is pointed out that genuine or classical cases are rare Evidence is produced to show that onchocerciasis may have definite arthropathic action, and it is suggested that cases of arthralgia so common in certain areas may be associated with latent onchocerciasis In relation to onchocercomata, an analysis of 87 cases shows the scalp to be affected in about 8 per cent of cases—a figure which contrasts sharply with the 40 per cent in South America Finally, it is regretted that such big gaps should exist in our knowledge on the nature of the disease, and further work on the pathogenesis of onchodermatitis is urged as one of the surest routes to the hitherto elusive specific against this troublesome malady

REFERENCES

ANDREWS, G C (1946) *Diseases of the Skin*, 3rd Ed Philadelphia and London W B Saunders & Co
BLACKLOCK, D B & SOUTHWELL, T (1940) *A Guide to Human Parasitology*, 4th Ed London H K Lewis & Co
FAIRLEY, N H (1946) *Price's Textbook of Medicine* Oxford University Press
GABATHULER, M J & GABATHULER, A W (1947) *E Afr med J*, 24, 188
GERMAN, W (1945) *Amer J trop med*, 25, 129
GOLDMAN, L (1944) *Arch Derm Syph*, 50, 385
——— & ORTIZ, L F (1946) *Ibid*, 53, 79
GOPSILL, W L (1939) *Trans R Soc trop Med Hyg*, 32, 551
HARRIS, B P (1940) *Ibid*, 34, 233
HAWKING, F (1939) *Ibid*, 33, 95
——— (1940) *Ann trop Med Parasit*, 34, 211
LOWENTHAL, L J A (1943) *Ibid*, 37, 147
MANSON-BAHR, P & MARTINS, P H (1939) *Trans R Soc trop Med Hyg*, 33, 8
——— (1945) *Manson's Tropical Diseases*, 12th Ed London Cassell & Co
——— (1944) *Synopsis of Tropical Medicine* London Cassell & Co
SCOTT, J G (1944) *Brit med J*, 2, 553
STRONG, R P (1945) *Stitt's Diagnosis, Prevention and Treatment of Tropical Disease*, 7th Ed London H K Lewis & Co

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MASS PROPHYLAXIS AGAINST SLEEPING SICKNESS IN SIERRA LEONE FINAL REPORT

BY

R D HARDING, D M, M R C P,

AND

M P HUTCHINSON, M R C S, L R C P

This paper embodies the findings 1 year after mass prophylaxis, chiefly with pentamidine isethionate, in two chiefdoms containing a population of over 12,000 It contains also a final note on the sleeping sickness position at 3 years in these two chiefdoms in which a second mass prophylaxis was given at 1 year, together with that in a third adjoining chiefdom containing 3,600 people which had received one initial prophylaxis only The total population dealt with over this long period is sufficient to demonstrate the value of the method on a large scale against the disease as met with in Sierra Leone

RÉSUMÉ OF PREVIOUS FINDINGS

As this report forms a sequel to a previous paper (HARDING and HUTCHINSON, 1948), our intermediate findings may be briefly recapitulated

Results in two areas were reported upon (1) The " Fuero area," where an atypical strain of *Trypanosoma gambiense* was causing an epidemic with various unusual characteristics, and (2) a "normal" area where the disease existed in the classical form normally met with in Sierra Leone In the Fuero area, 7 months after prophylaxis, no trypanosomes were found in blood or gland juice among 1,061 people who had received pentamidine isethionate mg 150 to 400, two blood positives were obtained among 437 people who had received antrypol gramme 1 (0 5 per cent), and 24 peripherally positive cases occurred among 471 untreated controls (5 1 per cent) Among the prophylactically treated people, 0 9 per cent revealed an abnormal C S F coupled with suggestive symptoms in the absence of demonstrable trypanosomes, probably most of these patients had already been suffering from an undiagnosed infection at the time of prophylaxis, but

the possibility that an occasional fresh infection, which had remained cryptic, might have occurred after prophylaxis could not be ruled out. In the normal area, in which the population was inspected at 2-monthly intervals, this time for 10 months no cases were found among 9[illegible]1 who had received pentamidine isethionate mg. 100 to 350 two peripherally positive cases were found by the end of this period among 772 people who had received antrypol gramme 1 to 2 (0·2 per cent.), and 22 were found among [illegible],407 controls (0·9 per cent.) There was no indication here of any cryptic infections having occurred.

To sum up: In 7 to 10 months after prophylaxis no overt infections had occurred in 1,982 people using pentamidine, four had occurred in 1,209 people with antrypol (0·3 per cent.) and 46 had occurred in 2,878 controls (1·6 per cent.). The great value of pentamidine as prophylactic and its superiority to antrypol in mass campaign was thus demonstrated, but it was decided that further period of observation was required to elucidate long-term results, and in particular to try to determine what danger if any existed from the possible occurrence of cryptic infections following prophylaxis.

FINDINGS AT 1 YEAR.

The normal area has not been followed beyond 10 months, and the present observations carried out 1 year after prophylaxis concern (1) The Fuero area previously reported on at 7 months and (2) a large region in Soa and Gbane Kando chiefdoms surrounding the Fuero area, and forming with it a solid block of country in which no full-scale intermediate examinations of the people had been carried out. In neither case had the population received any further drug since their prophylaxis a year previously (with the exception of some 500 original controls in the "Fuero area," who received pentamidine after 7 months observation). The method of examination was as follows Every person was carefully questioned as to his health, and any suggestive symptoms since his original injection cervical glands were felt for and any found were punctured, repeat punctures being done where more than one palpable gland existed two thick blood films were made from everybody stained with methylene blue and giemsa, and examined for 15 minutes lumbar puncture was performed and a C.S.F. cell count carried out if there existed the slightest indication from the history or appearance of the patient to suggest trypanosomiasis.

(1) *Fuero Area.*

Table I shows the findings after 1 year It excludes the few cases in the prophylactic groups diagnosed by blood film or altered C.S.F. at 7 months, but includes a few wh developed symptoms and sought advice at some time intermediate between 7 months and final examination. The group entitled "No prophylaxis comprised immigrants and others who had not been present at th time of prophylaxis. Their period of residence in the area prior to examination averaged about 6 months.

In addition to the people included in Table I there existed a group comprising the original controls used for the observations at 7 months reported

See map in our previous paper (HARDING and HUTCHINSON, 1948)

TABLE I —FUERO AREA—RESULTS 1 YEAR AFTER PROPHYLAXIS
(Percentages in brackets)

Drug	Adult dose	Population re-examined	Gland +	Blood +	C S F >5 cells
Antrypol	Gramme 1	480	0	2 (0 4)	1 (0 2)
Pentamidine isethionate	Mg 150 to 200	636	0	0	5 (0 8)
" "	Mg 375 to 400	586	0	0	4 (0 7)
No prophylaxis	—	360	5 (1 4)	3 (0 8)	2 (0 5)

in our previous paper Those not found infected at 7 months had each received pentamidine isethionate mg 175 (adult dose, children in proportion to body weight) at that time They had therefore been at risk for 5 months when re-examined The 423 people obtained for re-examination in this group gave no blood or gland juice positives and provided only two cases with abnormal C S F (0 5 per cent) They thus served to support our previous findings

(2) *Surrounding Region*

In this region the cases found before prophylaxis more generally resembled the common type met with elsewhere in Sierra Leone Table II shows the results 1 year after prophylaxis Though no untreated controls had intentionally been left in this region, a considerable number of people had immigrated from the surrounding country in the course of the year and had therefore received no drug, the last horizontal column entitled " No prophylaxis," is made up chiefly of these immigrants As with Table I, the cases revealed include some subjects who sought examination voluntarily at some intermediate period after prophylaxis

TABLE II —SURROUNDING REGION—RESULTS 1 YEAR AFTER PROPHYLAXIS
(Percentages in brackets)

Drug	Dose	Initial S S % 1945	Population re-examined	Gland +	Blood +	C S F >5 cells
Antrypol	Gramme 1	3 6	2,085	2 (0 1)	0	10 (0 5)
"	Gramme 1 × 2	2 2	348	0	1 (0 3)	2 (0 6)
Pentamidine isethionate	Mg 175 to 200	3 7	3,152	1 (0 03)	2 (0 1)	22 (0 7)
" "	Mg 375 to 400	3 6	1,259	1 (0 1)	0	9 (0 7)
No prophylaxis	—	—	2,624	48 (1 8)	4 (0 2)	16 (0 6)

The comparatively high rate of peripheral infection (2·0 per cent.) in the "No prophylaxis" group is evidence that the prophylactically treated people had been exposed to some risk of infection, as many of the immigrants had been living in the region for several months the same argument applies to the "No prophylaxis" group of Table I The proportion of immigrants in the Fuero and surrounding regions combined (strictly speaking, the term immigrants includes normal inhabitants of the region who had been travelling or residing outside it at the commencement of the year and some 400 infants born during the year as well as completely new arrivals from outside), is of importance in itself. These immigrants formed 25 per cent. of the total in a block of country about 200 miles square containing some 12,000 people This degree of coming and going is no unusual feature for Sierra Leone and reveals a mass of casual movement natural also to other parts of West Africa. Such movement makes it clear that no area in ordinary circumstances can be satisfactorily protected from re-introduction of the disease if it exists in the *surrounding country*

Summary of Results in Whole Area up to 1 Year In Table III are summarized the infections which have been revealed in the Fuero and surrounding regions combined over the whole year For the sake of conciseness, the various dosages of each drug are combined into single groups. It should be explained that the controls re-examined at 7 months have been included in the "No prophylaxis" group also that the cases revealed at 7 months in the antrypol and pentamidine groups have been added to those shown in Tables I and II.

TABLE III.—FUERO AREA AND SURROUNDING REGION COMBINED.
(Cases revealed in the course of 1 year following prophylaxis.)

Drug	Population re-examined.	Gland or blood —		C.S.F >3 cells.	
		Cases.	Per cent.	Cases.	Per cent.
Antrypol grammes 1 to 2	,913	7	0 24	17	0 59
Pentamidine isethionate mg. 150 to 400	5 653	4	0 07	49	0 7
No prophylaxis	3 463	84	2 4	1	0 61

There are certain points which should be emphasized in regard to the foregoing tables.

(1) Owing to the unusual characteristics of the disease in the Fuero area and, to a lesser extent, in the region surrounding it, which made it inevitable that a number of subjects who were already infected at the time of prophylaxis remained undetected and so received prophylactic injection, most of the cases ultimately diagnosed in the prophylaxis groups are attributable to pre-existing

infection It is even possible that all the cases revealed after pentamidine could be accounted for in this way some of the peripherally positive cases which had received antrypol were on the other hand undoubtedly new infections Owing to the suppressive effect of both drugs, it was to be expected that a number of missed cases would only reveal themselves later by the development of symptoms combined with an altered cerebro-spinal fluid Of the 44 cases diagnosed by abnormal C S F in the "surrounding regions" in the course of the year following prophylaxis, 18 (41 per cent) gave counts of over 100 cells, and 14 (32 per cent) of 20 to 100 cells, thus affording an indication that most had long-standing infections

(2) Results after prophylaxis are even better than appears at first sight by contrast with the unprotected subjects for two reasons Firstly, the latter had only been at risk for a period averaging some 6 to 7 months, as already explained, whereas the prophylactic groups had been at risk for 12 Secondly, the infection rate among the unprotected is very much lower than would have been the case if prophylaxis had not been undertaken among the remainder prophylaxis had the effect of putting the bulk of the trypanosomes out of circulation, so reducing the proportion of infected tsetse

(3) The higher or repeated doses of pentamidine conferred no advantages in protection over a single moderate dose (cp Tables I and II), and we consider an adult dose of mg 175 the optimum which combines effectiveness with freedom from serious side-effects for large-scale use

PRESUMPTIVE CRYPTIC INFECTION FOLLOWING ANTRYPOL

It is usually impossible to decide whether a case of the disease diagnosed after prophylaxis in the absence of demonstrable trypanosomes is a cryptic new infection or a pre-existing one For this reason the following case, which we believe to be an undoubted cryptic new infection though final proof of the infection is lacking, is worth recording

M K S, an intelligent native attendant on the staff of the campaign, was employed at times as a fly-boy in the course of the present work in investigating the tsetse distribution in the Fuero and surrounding areas He was given a prophylactic dose of antrypol gramme 1 in February, 1946 He remained in normal health until mid-November, when he suffered from an attack of fever and headache, the latter symptom being the more persistent On 20 11 46 he received pentamidine isethionate mg 150, after which his symptoms abated for about a fortnight but headache was complained of for a couple of days in early December On 23 12 46 severe headache with high fever commenced and did not respond to oral or intravenous quinine Temperature at first was swinging, but thereafter remained in the neighbourhood of 103° F Repeated blood examinations had proved negative and there was no glandular enlargement He received antrypol gramme 0 3 on 28 12 46 and gramme 0 8 on 30 12 46, but there was no improvement

On 1 1 47, by which time he was very thin and ill, and his temperature still 103° F, he received tryparsamide gramme 1 On 2 1 47, temperature was still 103° F, on 3 1 47, 101° F, with improvement in general condition, on 4 1 47 it had returned to normal and thereafter remained so He received a course of tryparsamide and made a complete and uneventful recovery From his response within 48 hours of his first dose of tryparsamide it was impossible not to believe that his recovery was due to this drug

It appears to us exceedingly probable that following antrypol prophylaxis in February 1946 this patient acquired a cryptic trypanosome infection (from the circumstances of his work and liability to infection very probably in May—he caught flies by allowing them to settle and feed on him, and a fly he caught in May was later proved by feeding experiments to be carrying infective *T. gambiense*), that the single dose of pentamidine in November produced temporary amelioration, but that the antrypol given at the end of December had no appreciable effect—possibly because his trypanosomes had acquired some resistance to this drug. It is unfortunate that owing to the existing circumstances of his becoming seriously ill in bush, animal inoculation or blood culture was not carried out; neither was lumbar puncture performed. But repeated clinical examination by both of us failed to suggest any other infection and no other disease was known to exist in the area which would have been likely to respond so dramatically to tryparsamide.

FINDINGS AT 3 YEARS.

Originally in 1945, mass prophylaxis had been given to the inhabitants of Soa, Gbane Kando and Mafindo chiefdoms (see map, HARDING and HUTCHINSON, 1948). Immediately following the re-examination of Soa and Gbane Kando a year later (these two chiefdoms contained the "Fuero area" and surrounding region of Tables I and II) a second mass prophylaxis had been undertaken therein, using entirely pentamidine isethionate in a dosage of mg. 175, but no examination or repeat prophylaxis of Mafindo was carried out at this time. Owing to shortage of staff, no further work was possible in either of the chiefdoms until the early part of 1949, when all three were re-examined under the direction of Dr. I. APTED, now in charge of the campaign, to whom we are greatly indebted for the 1949 figures shown in Table IV.

TABLE IV.—INCIDENCE OF SLEEPING SICKNESS [illegible] PROPHYLA[illegible] AND 3 YEARS LATER.

Chiefdom.	End 1943 S.S. per cent.	End 1945. Prophylaxis	End 1945. Dose.	End 1946. Prophylaxis	[illegible] Population examined	[illegible] per cent.
Soa	3 3	Antrypol pentamidine isethionate	Various	P I mg [illegible]	11 [illegible]50	[illegible]
Gb. Kando	7 8				[illegible]	[illegible]
Mafindo	1 7	Pentamidine isethionate	Mg. 175	Nil	3 6[illegible]	[illegible]
Whole area	3 5	—	—	—	[illegible]	[illegible]

Note.—Seventy-seven cases were found and treated in Soa and Gbane Kando during the re-examination of 1946 but from then until 1949 the only patients treated were such as voluntarily sought treatment at dispensary.

In this final examination only routine methods were used, *i e*, palpable cervical glands were punctured and if found negative blood films were examined, but blood films were not made on the whole population and no lumbar punctures were carried out However, the bulk of the cases diagnosed prior to prophylaxis and indicated by the incidence shown under the second column had been diagnosed by these routine methods—in fact, all except some of those in the comparatively small " Fuero area "—so the reduction in incidence is very real This is the more marked in that a number of the cases diagnosed in 1949 were immigrants from other chiefdoms From the previous history of the epidemic and the failure of its response to repeated surveys and treatment, plus the provision of dispensary facilities, it is clear that the great fall in incidence must be attributed to prophylaxis

Discussion

We believe the chief value of this report to lie in its being a record of a long-term follow-up of a sizeable block of country containing some 17,000 inhabitants in which the whole available population received prophylaxis Most other observers, *e g*, VAN HOOF *et al* (1946) and (1946a), BRUN-BUISSON (1947), FAIN and MULDER (1948), and LE ROUZIC (1948), using pentamidine or propamidine, have left a considerable proportion of their subjects untreated as controls during the period of observation, so allowing a human reservoir of the trypanosome to remain Our purpose was to show what would happen when every available person was so protected that already infected flies were likely to die out and the strain become lost before susceptibility to infection again returned Incidentally, our results indicate that in Sierra Leone there exists no serious reservoir other than man, though domestic animals, including cattle, goats, sheep, pigs and dogs are kept in most villages This indication is borne out to some extent by the examination of blood films which have been taken from some scores of pigs and cattle in areas of Sierra Leone where sleeping sickness was endemic, only one film—from a cow—revealed a polymorphic trypanosome, and about a dozen attempts to infect local pigs, whose blood was regularly examined for some months after, by inoculation from human cases gave negative results Such evidence is not decisive, but strongly suggestive

VAN HOOF *et al* (1944), using two volunteers, have shown that infection can occur 10 to 12 months after a prophylactic injection of pentamidine, and that such infections do not appear to follow the normal course We have been unable to prove that cryptic infections do not occur after pentamidine, though no evidence has arisen in the course of our observations that they do occur had they done so in any significant numbers, however, it is inconceivable they would not have revealed themselves at 1 year by a marked increase in the number of people with symptoms and an altered C S F, or at 3 years through ultimate peripheral relapse This question could probably only be decisively settled by repeated intensive investigation of a number of subjects which included

blood culture, animal inoculation, and C.S.F. examination prior to prophylaxis, and repetition of these procedures at intervals afterwards, on a scale which is impracticable in the course of a general campaign otherwise it is impossible to be certain that cases diagnosed subsequent to prophylaxis do not represent pre-existing undetected infections. However we think it permissible to conclude from the foregoing that the occurrence of cryptic cases of *T. gambiense* if indeed they do occur is not likely to be on a scale sufficient to contraindicate mass prophylaxis with pentamidine.

From the practical point of view the most important desideratum is the duration of protection of the great majority of subjects by one dose of pentamidine. If large communities can be protected for a whole year by a single injection mass prophylaxis possesses enormous practical value whereas if re-examination and re-injection were necessary every 6 months the procedure would not be nearly so practicable both because of the increased medical staff required and of the upset to the native's ordinary avocations—he would be liable to grow restless under such repeated interference when unaware of any illness.

On the basis of our own and others' recorded results we are of the opinion that the stage has been reached when it is justifiable to use mass prophylaxis with pentamidine in suitable epidemics on a large scale with a fair degree of confidence, with the proviso that it should be preceded by particularly careful diagnosis to discover and treat existing cases. We consider also that West African communities so protected may generally be safely left for a whole year after which a repeat examination should be carried out, normally followed by a second mass prophylaxis. Such repeat examinations should include a specially close scrutiny for subjects with suggestive symptoms or signs in the absence of peripheral trypanosomes and, if feasible, suspected cases should be lumbar punctured. The incidence should then have reached so low a level that dispensary facilities or occasional sampling surveys would suffice to ensure that any serious re introduction of infection does not occur. Very possibly a few individuals will crop up in whom, by reason of some personal idiosyncrasy or intercurrent disease the pentamidine is destroyed or eliminated from the system much more rapidly than in others, just as HAWKING (1940) has shown occur in the case of antrypol, but this possibility should not be given too much weight until proved. We are also impressed with the diversity of behaviour in man of the strains of *T. gambiense* met with in different parts of Sierra Leone and Nigeria, and would not be surprised if sooner or later an epidemic were met with in which pentamidine was found to protect for a much shorter time than normally. VAN HOOF *et al.* (1946a) note how much the nature of the strains they employed could influence the duration of protection by pentamidine in guineapigs. Finally it is possible that infections which do occur when a minimal amount of the drug remains in the body perhaps after a year may eventually give rise to the propagation of pentamidine resistant strains. LOURIE and

YORKE (1938) and FULTON and YORKE (1941) have shown that *T rhodesiense* can be rendered resistant to a diamidine, though it is a slow process and the resistance acquired lapses with time, and there appears to be no work on record to indicate whether such resistance is cyclically transmissible. We consider that, though these possibilities should be borne in mind, in view of the great advantages of pentamidine prophylaxis in suitable circumstances they should not act as a deterrent in its wide-scale employment

SUMMARY

1 This report concludes the account of a large-scale experiment in mass prophylaxis against sleeping sickness using chiefly pentamidine isethionate but with some antrypol for comparison, and forms the sequel to a previous paper reporting intermediate results at 7 months in a part of the area concerned where an unusual type of the disease occurred These intermediate results are briefly recapitulated

2 The findings at 1 year are given for the "Fuero area," the subject of our previous report, together with those of the surrounding region The protected population re-examined numbered some 8,500, while 3,450 "immigrants" who had not been protected were also examined for comparison A peripheral infection rate of 0 07 per cent was found among subjects who had received pentamidine and one of 0 24 per cent among those who had received antrypol, both in various doses The respective figures for subjects presumptively diagnosed by a raised C S F cell count were 0 87 per cent and 0 58 per cent Among the immigrants 2 4 per cent revealed peripheral trypanosomes and 0 61 per cent. a raised cell count

3 It is believed that most, if not all, of the cases found at 1 year among the subjects who had received pentamidine were already infected though undetected when they received the drug An occasional case among those found infected after antrypol appeared, however, to be recent

4 The optimum prophylactic dose of pentamidine isethionate decided on from the viewpoint both of efficiency and freedom from side-effects was mg 175

5 A presumptive case of cryptic infection following antrypol is described

6 The population re-examined at 1 year received a second prophylactic injection at that time of pentamidine isethionate mg 175 Figures have now been sent us showing the infection rates obtained rather more than 2 years later in the two chiefdoms concerned, and also in an adjoining chiefdom which received initial prophylaxis only and then was left untouched for 3 years The overall incidence found was about one-fifteenth of that prevailing in 1945 just prior to prophylaxis

7 The implications of our findings are discussed and various theoretical drawbacks to mass prophylaxis mentioned It is concluded that our own and others' results justify the use of pentamidine prophylaxis on a wide scale in

suitable epidemics provided that special care is taken to diagnose and treat the maximum number of existing cases before prophylaxis, and that at subsequent examinations a watch is kept for cases with suggestive symptoms and signs in the absence of demonstrable trypanosomes. We recommend in general that prophylaxis should be repeated after 1 year followed by surveillance through occasional sampling surveys or stationary dispensaries.

REFERENCES.

BRUN-BUISSON, P, DIALLO, J, PIRION, L. & CAILLE, J (1947) *Bull. Méd. Afr. O. F.* 4, 257

FAIN, A. & DE MULDER, P (1948). *Ann. Soc. belge Méd. Trop.* 28, 161

FULTON, J. D. & YORKE, W (1941) *Ann. trop. Med. Parasit.* 35, 221.

HARDING, R. D. & HUTCHINSON, M. P (1948). *Trans. R. Soc. trop. Med. Hyg.* 41, 481

HAWKING, F (1940) *Ibid.*, 34, 37

LE ROUZIC, J (1948). *Bull. Méd. Afr. O. F.* 5, 89

LOURIE, E. M., & YORKE, W (1938) *Ann. trop. Med. Parasit.* 32, 201

VAN HOOF, L., HENRARD, C. & PEEL, E. (1944). *Trans. R. Soc. trop. Med. Hyg.* 37, 271

——— ——— ——— (1946) *Ann. Soc. belge Méd. Trop.*, 26, 371

——— LEWILLON, R., HENRARD, C., PEEL, E. & ROODENVENKET, B. (1946a). *Trans. R. Soc. trop. Med. Hyg.* 39, 327

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TOXIC ACTION OF EMETINE ON THE CARDIOVASCULAR SYSTEM *

BY

A D CHARTERS, M D (CAMB), M R C P, D T M & H,
Late Major, Medical Specialist

It is essential, when dealing with such a widely prescribed drug as emetine, to acquire as much knowledge as possible about its dangers and limitations The opportunities offered by military conditions prompted me to perform these investigations, which were carried out in a military hospital in Kenya during the year 1944 Eleven cases (ten East Africans and one Sikh) were examined Nine of the patients were suffering from amoebiasis and two from schistosomiasis with post-arsenical jaundice An examination of each patient was performed before and immediately after the completion of a course of 12 daily intramuscular injections of emetine grain 1 Nine of the patients were again seen after a further interval, varying from 14 to 41 days The investigations were as follows

(1) Tests for interference with venous return, by means of a clinical examination for venous congestion of the cervical veins, oedema of the legs, enlargement of the liver, pulmonary congestion and albuminuria

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*I am indebted to Professor (then Lieut -Colonel) A Kerwick for his valuable advice and encouragement, to Dr (then Major) W G Hopkirk for kindly performing the radiological investigations, and to Majors Cam, Gellert and Wright for allowing me to examine their patients I am grateful to Brigadier J M Macfie, C B E, M C, D D M S East African Command, for permission to publish this article

(2) Tests for circulation rate by means of arm to tongue estimations with 10 per cent. calcium gluconate and arm to lung determinations with 5 minims of ether and 5 minims of normal saline. The normal average circulation rate for calcium gluconate was found by BARR (quoted by P D WHITE, 1944) to be 12 3 seconds (ranging from 8 to 16·5 seconds), and for ether 5·8 seconds (ranging from 3 to 9 seconds). BARR employed 4 c.c. of 20 per cent calcium gluconate but as this concentration was unavailable rapid injection of 10 per cent. solution was employed. In order to test the efficacy of these methods the circulation time of case of pericardial effusion, suffering from congestive failure was estimated the patient figures were 16·? seconds for calcium gluconate and 7 seconds for ether

(3) Blood pressure examinations.

(4) Examinations for cardiac dilatation.

(a) Clinically by palpation of the apex beat and percussion of the cardiac dullness and

(b) Radiologically by comparison of tracings of the cardiac outline during screening before and after the course

(5) Counts of the resting pulse rat

(6) Exercise tolerance tests, which consisted of stepping ten times on to bench 18 inches high.

(7) Electrocardiography Owing to shortage of films, the three classical leads were alone employed.

The results are tabulated below

There was no evidence either clinically or radiologically in any of the patients, of cardiac dilatation or impairment of venous return.

EXAMINATION IMMEDIATELY AFTER EMETINE COURSE.

(1) *Circulation Time* The circulation time which was estimated in eight cases of this series, was increased in three decreased in four and unchanged in one. The results are inconclusive. Depending as they did on the African's intelligence the tests were somewhat unreliable.

(2) *Blood Pressure* All 11 patients were tested. The systolic pressur fell after emetine in ten cases (by over 9 mm. in five cases), the average fall being 8 mm. and the maximum drop 24 mm. The average systolic pressur before emetine was 128 and after therapy 118 mm. Hg The systolic pressure was raised by 16 mm. in one case, without any accompanying change in diastolic pressure. The diastolic pressure dropped in six cases (by over 5 mm. in four cases), the average fall being 4 mm. and was unchanged in five cases The average diastolic pressures before and after the injections were 79 mm and 75 mm. respectively

If we adopt the formula $\text{Diastolic} = \dfrac{\text{Systolic} + 20}{2}$ (WILKINSON 1947), the average diastolic pressures, both before and after the drugs, are 6 mm above that expected for the corresponding average systolic pressures.

(3) *Exercise Tolerance Test* This was worse in seven cases better in one case (Case 3) (a patient who was very ill with pyrexia, hepatitis and acti

Entamoeba histolytica in his stools before the injection, and whose electrocardiogram improved along with his exercise tolerance and general physical condition), and unchanged in two cases It was not performed in the case of one patient who was suffering from an amoebic abscess of the liver and who was too ill for the test

(4) *Resting Pulse Rate* This was more rapid in eight cases, slower in two cases (one was the toxic case mentioned above and the other patient was suffering from dysentery, vomiting and cough before treatment), and unchanged in one The average rise in rate was six beats per minute

(5) *A systolic murmur* appeared after emetine in one case, and in two cases a systolic murmur, present before therapy, became louder after the completion of treatment

(6) *Electrocardiography* Three cases showed slight abnormalities of the electrocardiogram before administration of the drug, presumably as the result of toxic action of the disease All three were improved both clinically and electrocardiographically after their treatment Thus, one of these patients (Case 3) who was suffering from pyrexia, hepatitis and dysentery, with active *E histolytica* in his stool, had an inverted T2 and a raised S-T interval on admission which had become upright and isoelectric by the end of his course The second patient (Case 4), who was also suffering from toxic symptoms —vomiting, dysentery and cough—showed flattening of T2 before treatment, which became upright after his injections The third patient (Case 8) had flattening of T in all three leads before therapy and a more upright T1 and T2 after his course

There was a deterioration of the electrocardiogram at the end of the emetine course in six cases (all of which had normal electrocardiograms before treatment), although evidence of definite myocardial disease, as revealed by inversion of T in all leads, was only present in one case The other five cases showed flattening of T in all leads (one case), flattening of T in leads 1 and 2 (two cases), flattening of T in lead 2 only (one case), and a slightly lower voltage curve (one case)

No abnormality of auriculo-ventricular or intraventricular conduction was observed, neither was there any interference with the normal sinus rhythm There was no sign, in this series, of emetine poisoning elsewhere in the body

SUBSEQUENT EXAMINATIONS

Nine of the patients were re-examined later, at intervals varying from 14 to 41 days (average 32 days) from the last injection

(1) *Blood Pressure* Of the nine cases examined, six showed a rise of pressure, which involved both systolic and diastolic pressures in four, systolic pressure alone in one, and diastolic pressure alone in one The systolic and

diastolic pressures rose to (or exceeded) the same level as before treatment in three cases. There was a still greater fall (over and above the drop in pressure immediately after the emetine course) in both systolic and diastolic pressures in three cases, after 32, 14 and 16 days respectively

The average systolic and diastolic pressures were 120 and 76 mm. respectively a rise of only 2 mm. in the systolic and 1 mm. in the diastolic pressures over the average figures at the termination of the emetine injections. The absence of a definite rise in average pressure is partly explained by the fall which occurred in the pressures of two cases after 14 and 16 days respectively before the cumulative action of the drug had time to wear off

(2) *Exercise Tolerance Test* Seven cases were examined. There was an improvement in five cases, to the same level as before treatment in one patient and to an even better degree in four Of the remaining two the test was unchanged in one and slightly worse in the other

(3) *Resting Pulse Rate* Out of nine cases there was a decrease in rate in five cases, to the same level as before therapy in two, and to an even slower rate (presumably due to the beneficial effect of treatment) in three. The pulse was unchanged in two cases, and was slightly more rapid in two cases (one patient was suffering from post arsenical jaundice and urinary schistosomiasis). The average rates before emetine immediately after the course and after an interval averaging 32 days from the last injection, were 80 86, and 80 beats per minute respectively It will be seen that the average pulse returned to its rate before the commencement of treatment.

(4) *Electrocardiography* There was an improvement in the electrocardiograms of four of the six cases which had deteriorated after the emetine injections. Thus, the patient (Case I) with inversion of T in leads 1 2 and 3 had after 41 days, acquired an upright T1 (T2 and T3 remaining still inverted). T1 and T2 became upright in the three others who had previously shown flattening of these waves. Only one patient's electrocardiogram was worse (24 days after his last injection) his T2 had become inverted, his T1 had become more flattened and a deep Q3 had appeared. The patient (Case 11) was suffering from an amoebic abscess of the liver which was being drained. His myocardial disease can probably be ascribed to toxic absorption, rather than to the cumulative action of emetine.

EMETINE OVERDOSAGE.

In 1943 I saw two patients, both of whom had been accidentally over dosed. One had received 20 grains and the other 32 grains in consecutive daily intramuscular injections. Both patients were undergoing treatment for amoebic dysentery and both had ancylostome ova in their stools. The first patient, a Nyasa native, was seen 2 weeks after the termination of his 20 injections.

His resting pulse rate was 102 per minute, blood pressure systolic 146 mm and diastolic 96 mm Hg, exercise tolerance, 102/144/108, circulation time 10 seconds (calcium gluconate) and 4 9 seconds (ether), no cardiac dilatation, venous congestion or albuminuria An electrocardiogram, performed 5 weeks after his last emetine injection, showed a low voltage curve, no deflection in any lead exceeding 4 mm and QRS of lead 2 not projecting over 2 mm from the base line in any direction No other abnormality was present in the films A second electrocardiogram, taken 8 weeks after the first tracing (13 weeks after the last injection), revealed a return to normal voltage At this time his pulse was only 72, blood pressure, 150/100, exercise tolerance, 72/108/72 The patient made an uninterrupted recovery At no time did he show any skin eruption, abnormality of nervous system or disturbance of gastro-intestinal function

The second case, an African of the Acholi tribe, was first seen immediately after the termination of his 32-grain emetine course He had a sparsely-distributed papular rash on thighs, calves, chest and abdomen, accompanied by a generalized dry, scaly, irritating dermatitis, most marked on the extensor surfaces He was suffering from diarrhoea His resting pulse was 84 Blood pressure, 122/78 Exercise tolerance test, 84/96/84 Circulation time, 12 9 seconds (calcium gluconate) An electrocardiogram, 3 weeks after his last injection, showed no abnormality No venous congestion, cardiac dilatation or albuminuria There were no neurological signs No parasites found in his stool The patient made a steady recovery His diarrhoea cleared up within a week of discontinuing his injections and the dermatitis disappeared after 3 weeks

That considerable tolerance to emetine is possessed by certain people is revealed by the case of a European settler in Kenya, who reported that he had received over 120 injections (varying from grain ½ to 1) of this alkaloid during the 4 years 1935 to 1939 The drug was administered in courses of 5 to 10 injections at intervals of a few months The patient must have received an average of over 20 grains of emetine per year He suffered from no diarrhoea or other toxic sign, and no abnormality could be found on examination of his skin, nervous system or cardiovascular system He was probably saved from toxic symptoms by the intervals between courses

Discussion

Histological signs of myocardial damage following emetine administration have been provided both in man and animals (Anderson and Leake, 1930, Chopra, Ghosh and De, 1924, Hein and Vannotti, 1939, Rinehart and Anderson, 1931, Epstein, 1932)

Several workers have supplied convincing evidence of the cardiac action of emetine on experimental animals Levy and Rowntree (1916) demonstrated by means of the electrocardiograph that overdosage of rabbits with emetine causes death by auricular fibrillation Boyd and Scherf (1941) studied the electrocardiographic changes in cats and dogs after intravenous injection of emetine They found that the commonest alteration was a prolongation of intraventricular conduction Bradycardia, prolongation of auricular-ventricular conduction, upward deflection of T waves (normally inverted in these animals), auricular extrasystoles and paroxysmal auricular tachycardia also occurred Ventricular extrasystoles and paroxysmal ventricular tachycardia were produced by advanced stages of intoxication, were usually terminal and were frequently

antecedents to ventricular fibrillation. Cardiac dilation, especially involving the right ventricle, occurred in association with the widening of the ventricular complex and cleared up concurrently with the improvement of intraventricular conduction. The authors observed that since the alterations which follow intravenous injections of the drug rapidly disappear there is reason for believing that these changes would not develop if an equal amount of emetine hydrochloride was subcutaneously administered. The cumulative effect of the drug in animal experiment is proved by BOYD and SCHERF's observation that second or third injections provoked progressively greater effects, even after apparent recovery of the electrocardiogram from the first injection, and by the findings of WALTERS and KOCH (1917) who noted that doses only one-tenth of the minimal single fatal dose became fatal when repeated daily for 3 weeks.

When we come to study the toxic action of the drug in man we find less unanimity of opinion. NAPIER (1943) states that the most dangerous and important effect is on the heart, in which it produces myocardial degenerative changes and alterations in conductivity with a fall of blood pressure cardiac irregularity and acute dilatation as the result of any undue effort. Many physicians, including CHOPRA and CHOPRA (1947) claim that emetine is a cardiac depressant, while CHOPRA (1934) and MACKIE (1936) warn against its use in organic heart disease. DACK and MOLOSHOK (1947) describe nine cases of toxic manifestations following emetine therapy. The dosage ranged from gram 7 to 22, administered over varying periods with, in the case of the larger dosages intermissions in treatment. The dosage required to produce toxic cardiac effects differed in each case, one patient revealing electrocardiographic changes after only 4 grains. The commonest electrocardiographic abnormalities consisted of flattening or inversion of T waves in a variable number of leads. Other changes, such as slight depression of S-T segment, deep Q waves and W shaped QRS were occasionally seen. Tachycardia praecordial discomfort dyspnoea and fatigue on exertion also occurred, but there was no significant lowering of blood pressure or abnormality of cardiac size. Toxic effects on the neuromuscular and gastro-intestinal systems generally preceded the appearance of toxic cardiac signs.

BROWN (1935), on the other hand, recording the results of emetine therapy on 554 cases of amoebic dysentery at the Mayo clinic, found not a single reference to cardiovascular disturbance. The dose which varied in individual cases, averaged gramme 0·65 (grain 10). Only 16 or 2·8 per cent. of the whole series exhibited an emetine reaction. Only three of the 16 reactions (0·5 per cent of the whole series) occurred at a dose of grain 10 or less. BROWN was able to find reports of only ten deaths attributed to emetine in the period from 1912 to 1935. Eight of the deaths had occurred after a total dosage of over grain 15. One of the two remaining cases was a child of two who died after single injection of gramme 0·02. HELLIO and VAIDYESWAR (1943) administered course of 12 daily intramuscular injections of emetine grain 1 to 14 patients

with 1 day's interval after the sixth injection Electrocardiograms were taken before the course, after the sixth and again after the last injections No deterioration of the electrocardiogram occurred in any case, with the exception of one patient who continued to suffer from dysentery and fever up to the tenth injection, and whose tracing at the end of the course showed an improvement on that after the sixth injection HARDGROVE and SMITH (1944) treated 72 patients by means of ten daily injections of grain 1, with only one serious cardiac complication, but 53 per cent of the patients developed minor electrocardiographic changes, the vast majority affecting the T waves, though actual inversion of T occurred in only 13 per cent COTTRELL and HAYWARD (1945), employing a similar dosage, observed flattening or inversion of T in one or more leads in 25 out of 32 cases

The majority of the 11 cases described in this article showed slight deterioration of cardiovascular function after 12 daily intramuscular injections of emetine grain 1, the systolic and diastolic pressures being depressed in ten and six cases respectively, the pulse rate being increased in eight, the exercise tolerance being worse in seven and the electrocardiogram showing deterioration in six

The discrepancy between the results of various workers probably arises, partly from individual idiosyncrasy to the drug, but mainly through differing dosages, for instance, BROWN, HARDGROVE and SMITH, HEILIG and VISVESWAR, and the author of this paper, all employed a total dosage of grain 10 to 12, with only minor evidence of cardiovascular impairment The toxic effects recorded by DACK and MOLOSHOK, on the other hand, occurred with dosages of grain 14 to 22 in two-thirds of their cases NAPIER also stresses the danger of overdosage He states " During the 1914–18 war, the writer saw many examples of inexperienced medical officers giving two and even three grains of emetine daily for long periods and literally killing their patients, of whose fate they were often quite unaware on account of the frequent evacuations from hospital that are inevitable in war time " There is no doubt that the therapeutic closely approximates to the toxic dose An *Indian Medical Gazette* editorial (1943) states " The single therapeutic dose of 1 grain is, in a 10-stone man, 1 milligram per kilo and is well within the limit of safety from toxic effects " BROWN's large series of 554 cases, with only 0 5 per cent of reactions at a total dosage of grain 10 or less, supports the adoption of this as a maximum dosage The majority of fatalities have occurred with total dosages of over grain 15

The factor of individual tolerance in determining susceptibility or resistance to toxic effects is illustrated, not only by three cases recorded in this paper, but also by BROWN's account of three patients who received grain 125, grain 134, and grain 180 respectively in a period of from 8 to 12 months without sign of intoxication

It is interesting to note that three of the writer's patients showed slight electrocardiographic abnormalities before the onset of treatment, which

improved after emetine administration. A similar observation was made by HEILIG and VISVESWAR, who found that 34 out of 45 patients exhibited pathological electrocardiograms before the commencement of treatment; the abnormalities, which consisted of low voltage curves, flat T waves or rarely depressed S-T segment, improved after intramuscular emetine in 11 out of 14 cases. The explanation of these observations seems to be, in the words of HEILIG and VISVESWAR, "That the positive effect upon the heart, exerted by the improvement of the intestinal and general condition under the influence of emetine, prevails over a possible negative effect on the heart muscle. There is no doubt that amoebic dysentery increases the permeability of the colonic mucosa to such an extent that an amount of intestinal toxins—though not of amoebic origin—sufficient to damage the myocardium enters the circulation."

The well known cumulative effect of emetine is not illustrated in this paper except perhaps by the slight fall of blood pressure which appeared in two cases (Cases 10 and 9) 14 and 16 days respectively from the termination of treatment, and by the slight deterioration of exercise tolerance exhibited by one patient (Case 7) 41 days after his last injection. Such action has, however, been demonstrated both by animal experiment, as described above, and by the work of DACK and MOLOSHOK, who found that the electrocardiographic abnormalities were not only often delayed for 1 or 2 weeks following the discontinuation of treatment (one case showing the first change 3 weeks after the last injection) but were also of long duration, persisting for periods varying from 1 to 4 months. The cumulative action has been attributed to slow excretion of the drug through the kidneys and intestinal tract (DACK and MOLOSHOK). MATTEI (1920) found emetine present in the urine 60 days after an 8-day course of gramme 0·48. DACK and MOLOSHOK suggest that the long duration of the electrocardiographic abnormalities found in their cases results from prolonged fixation of the drug in the myocardium or actual myocardial degeneration. In view of these findings it is advisable that an interval of 2 or 3 months should be allowed to elapse between courses of emetine. CHOPRA (1936) recommends an interval of 3 months. DACK and MOLOSHOK advise that a space of 1 or 2 months should be permitted, but that "if significant electrocardiographic changes or other clinical evidence of toxicity are observed during or after the first treatment with emetine hydrochloride, at least 2 months should be allowed to intervene before further emetine hydrochloride is administered."

SUMMARY

1. Eleven patients were examined before and immediately after a course of 12 daily intramuscular injections of emetine grain 1. Nine of the patients were re-examined after an average interval of 32 days from the last injection.

2. There was an average fall of 8 mm. systolic and 4 mm. diastolic pressure after the course.

3 The exercise tolerance deteriorated in seven out of nine cases after the emetine injections, but returned to the previous level in five out of the seven cases after a further average interval of 32 days

4 The average resting pulse rates before emetine, immediately after the course and after the subsequent average 32 days' interval, were 80, 86 and 80 respectively

5 The electrocardiogram deteriorated after the injections in six cases, one showing inversion of T1 and T2, four displaying flattening of T (in all leads in one case, in leads 1 and 2 in two cases, and in lead 2 only in one case), and one displaying a lower voltage curve Four of the patients showed an improvement when re-examined after the interval

6 Three patients showed slight abnormality of the electrocardiogram before the onset of treatment, presumably as the result of toxic action of the disease (two were severely ill before the injections) All these patients improved, both clinically and electrocardiographically, after the emetine, T waves, which had previously been inverted or flattened, becoming upright, the pulse becoming slower (two cases) and the exercise tolerance undergoing improvement (one case) Also of toxic origin were the inverted T2 and deep Q3 in the electrocardiogram of a case of liver abscess, taken 24 days after his last emetine injection

7 Two cases of accidental emetine overdosages are described, the patients having received grain 20 and 32 respectively in consecutive daily injections of grain 1 The first was suffering from myocardial disease, as evidenced by tachycardia, lowered blood pressure, poor exercise tolerance and low voltage electrocardiogram, all of which cleared up within 13 weeks of the last injection The second had diarrhoea, which ceased after a week, and dermatitis, which disappeared within 3 weeks

8 The case is described of a man who received 120 injections (probably over 80 grains of emetine) during the space of 4 years without toxic signs

9 No case of cardiac enlargement, irregularity of rhythm or interference with conduction followed intramuscular emetine injections, though such signs have been reported in animals after intravenous injection of the drug

13 The literature has been discussed

REFERENCES

ANDERSON, H H & LEAKE, C D (1930) *Amer J trop Med*, 10, 249
BOYD, J L & SCHERF, D (1941) *J Pharmacol*, 71, 362
BROWN, P W (1935) *J Amer med Ass*, 105, 1319
CHOPRA, R N (1934) *Ind Med Gaz*, 69, 309
——— (1936) *A Handbook on Tropical Therapeutics* Calcutta Art Press
——— & CHOPRA, I C (1942) *Ind Med Gaz*, 77, 65
———, GHOSH, B N & DE P (1924) *Ibid*, 59, 338
COTTRELL, J D & HAYWARD, G W (1945) *Brit Heart J*, 7, 168

DACK, S & MOLOSHOK, R. E. (1947). *Arch intern Med* 79 228
EPSTEIN, D (1932). *Quart J Pharm.*, 5 21
HARDGROVE, M. & SMITH E. R. (1944). *Amer Heart J* 28, 75_
HEILIG, R. & VISVESWAR, S. K. (1943) *Ind. Med. Gaz* 78 419
HEIN, G & VANNOTTI, A. (1939) *Z ges. exp Med* 104 629
Ind Med Gaz (1943). 78 443 Editorial.
LEVY R. L. & ROWNTREE, L. G (1916) *Arch. intern Med* 17 420
MACKIE, T T (1936) *Amer J digest. Dis & Nutr* 3, 566
MATTEI, C. (1920). Quoted by BROWN, P W (1935) *J Amer med. Ass* 105 1319
NAPIER, L. E. (1943). Quoted by *Ind. Med Gaz* (1943) 78 443 Editorial.
RINEHART J F & ANDERSON, H. H (1931) *Arch. Path* 11 546
WALTERS, A. L. & KOCH E. W (1917) Quoted by SOLLMANN, T (1947) *A Manual of Pharmacology and its Applications to Therapeutics and Toxicology* Philadelphia W B Saunders.
WHITE, P D (1944). *Heart Disease* p 238 3rd Ed. New York Macmillan & Co
WILKINSON K. D (1947). *The Practitioner* 158 431

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TRICHOSPOROSIS (PIEDRA) IN MALAYA

BY

R GREEN, M D , D SC ,

AND

D S MANKIKAR, M B , B S

(From the Institute for Medical Research, Kuala Lumpur, Malaya)

Trichosporosis is a condition in which hard nodules adhere to the hair shaft These are spore masses of a fungus *Trichosporon* or of a separate genus *Piedraia* This hair disease is found in equatorial America and in Asia and Europe Trichosporosis has been known to occur in Malaya since 1936 *

Trichosporosis usually affects the hair of the scalp, but has also been found on the eye-lashes, beard and moustache of men The nodules may be very minute or as large as the head of a pin, and may vary in colour from white to grey to black They are mostly elongate or oval-shaped and adhere tenaciously to the shaft of the hair as hard concretions , hence the term *piedra* (stone) Combing the hair, according to SAVILL (1935), may in consequence be a noisy process Under the microscope, after having been softened by soaking in liquor potassae, the nodules are seen to consist of spores which are closely adherent to one another and seemingly embedded in a viscous or cement-like substance

Material from the first case described in Malaya was referred to us by NIVEN (1936) Later, FASAL (1939) submitted specimens from three cases All four cases occurred in male Europeans between the ages of 30 to 35, and from Malayan cases seen so far, morphological findings have been similar Dr J W FIELD (personal communication) saw one case in an interned male European during the period of the Japanese occupation

* In South-East Asia, cases of Trichosporosis have also been reported from India and Ceylon by CASTELLANI and CHALMERS (1910), from Borneo by KUYPERS (1936), MANSON-BAHR (1945), from Indo-China by SOUCHARD and NGUYEN-VAN-HUONG (1937), and from Batavia by LAMPE (1940) The spores seen by LAMPE (1940) measured about 5μ, and are similar in size and shape to those encountered in Malaya

DACK, S. & MOLOSHOK, R. E. (1947). *Arch. intern. Med.*, 79, 228.
EPSTEIN, D. (1932). *Quart. J. Pharm.*, 5, 1.
HARDGROVE, M. & SMITH, E. R. (1944). *Amer. Heart J.*, 28, 752.
HEILIG, R. & VISVESWAR, S. K. (1943). *Ind. Med. Gaz.*, 78, 419.
HEIN, G. & VANNOTTI, A. (1939). *Z. ges. exp. Med.*, 104, 629.
Ind. Med. Gaz. (1943). 78, 443. Editorial.
LEVY, R. L. & ROWNTREE, L. G. (1916). *Arch. intern. Med.*, 17, 420.
MACKIE, T. T. (1936). *Amer. J. digest. Dis. & Nutr.*, 3, 566.
MATTEI, C. (1920). Quoted by BROWN, P. W. (1935). *J. Amer. med. Ass.*, 105, 1319.
NAPIER, L. E. (1943). Quoted by *Ind. Med. Gaz.* (1943). 78, 443. Editorial.
RINEHART, J. F. & ANDERSON, H. H. (1931). *Arch. Path.*, 11, 546.
WALTERS, A. L. & KOCH, E. W. (1917). Quoted by SOLLMANN, T. (1942). *A Manual of Pharmacology and its Application to Therapeutics and Toxicology.* Philadelphia: W. B. Saunders.
WHITE, P. D. (1944). *Heart Disease*, p. 238, 3rd. Ed. New York: Macmillan & Co.
WILKINSON, K. D. (1947). *The Practitioner*, 158, 451.

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Trichomycosis resembles trichosporosis. The former gives rise to soft yellowish excrescences which appear usually on the hairs of the axilla and pubis. Depending on a symbiotic association with cocci the colour assumed by the excrescence may be also red or black. Microscopic examination, however shows the presence of mycelium, whereas in trichosporosis the hard ovoid nodules consist entirely of spores apparently embedded in a cement like substance which prevents separation of the spores when compressed under a cover-slip The size and shape of the spores vary among the different species of *Trichosporon* as occurring in various countries and these species, according to BYAM and ARCHIBALD (1923), may be differentiated as follows

( ) Spores, polyhedral

(1) *Diameter 12µ to 15µ found in Columbia (T giganteum* BEHREND, 1890).

(2) Diameter 3 t 4µ found in Europe (*T beigeli* RABENHORST 1867)

(*b*) Spores, oval and small

(1) Hyphae in cultures twisted like corkscrew found in Europe (*T ovale* UNNA, 1896)

(2) Hyphae in cultures not so twisted, found in Europe (*T ovoides* BEHREND 1890)

(*c*) Spores, roundish

In symbiosis with coccus found in Europe (pubic hair of diabetic) (*T glycophile* DU BOIS, 1910)

MOORE (1944), however in a general account of piedra differentiates clearly a further genus of an organism occurring in South America which produces piedra, named *Piedraia hortai* (Brumpt) Fonseka and Area Leão 1928 and he makes a distinction as follows

	Trichosporon giganteum	*Piedraia hortai.*
Principal locality	Colombia.	Brazil.
Colour of nodule	White, cream, or brown	Grey brown, or black
Consistency	Soft or hard and brittle sometimes mucoid en velope	Hard, brittle
Spores	Spherical, thick-walled	Rectangular
Size	Some 10µ to 12µ, with many multilocular thick-walled larger cells	Approximately 7µ t 12
Asci	None	Ovoid
Ascospores	—	Eight 40 6µ
Cultures on Sabouraud agar	Yellowish rugose	Small, r secon.

FIG 1—Piedra nodules on heavily infected hairs

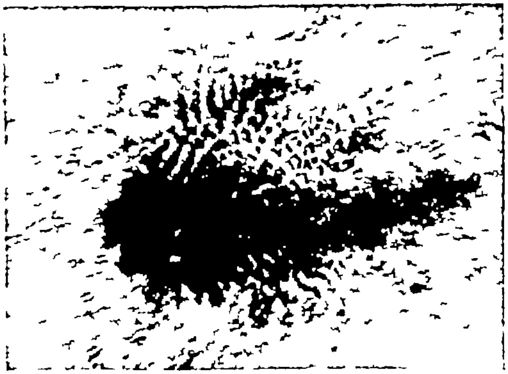

FIG 2—Early beginning of nodule formation on shaft of hair

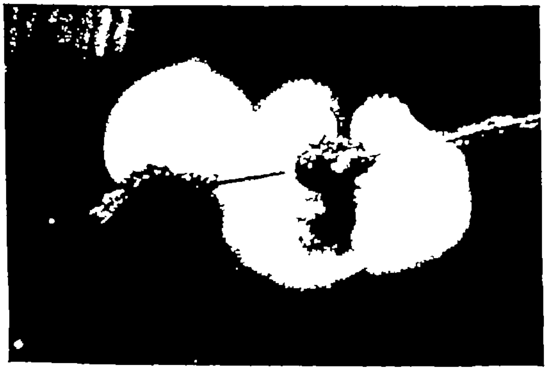

FIG 3—Cultures on Emerson's agar (3 days) showing colonies of associated cocci The nodule on the left is beginning to sprout

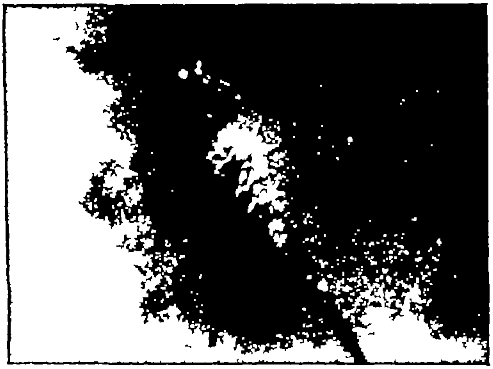

FIG 4—Cultures on Emerson's agar (5 days) Mycelium is sprouting from the nodule

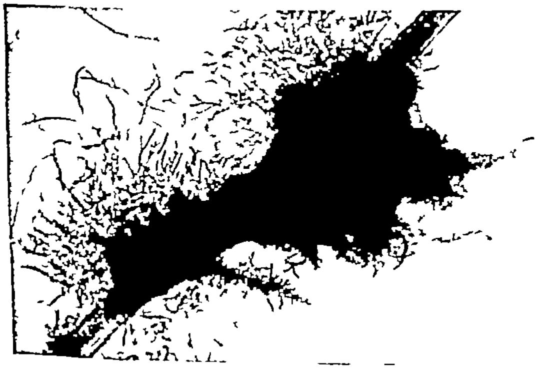

FIG 5—A sprouting nodule removed from an agar culture and viewed by transmitted light

FIG 6—Mycelium from a portion of the specimen shown in FIG 5

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Fig. —Cultures on Emerson agar (8 d). Mycelium developing from spores.

Fig. 8.—Cultures on Emerson agar (3 days). Type and structure of mycelium.

Fig. 9.—Piedra nodule immersed in Emerson fluid culture medium (3 days). Mycelium is not formed under these conditions but instead there is development of asci which occur at regular intervals inside the spore mass.

Fig. 10.—A in Fig. 9. Mass of polyhedral spores with regularly spaced asci within.

Fig. 11.—Asci seen more clearly after compression of the specimen.

Fig. 12.—Asci each containing eight elongate ascospores within.

The Organism causing Trichosporosis in Malaya

The nodules from cases of piedra so far found in Malaya have shown polyhedral or rectangular spores (see Figs 1, 2 and 10), measuring 2 2μ to 8μ, with a general average of 4μ or 5μ for different specimens

When the piedra nodules are placed on Emerson's agar*, the spores sprout and grow luxuriantly within 5 days at room temperature (25 to 28° C) (Figs 4, 5, 6, 7 and 8)

On the other hand, if the piedra nodules are immersed in culture fluids for 3 days, or if they are kept in a moist chamber for a week or more, or, again, in mucilaginous proprietary hair fixatives, a series of asci develop inside the nodules at regular intervals within the spore mass (Figs 9, 10 and 11) These asci, which measure up to 30μ in the long diameter, contain eight elongate ascospores (Fig 12)

The resemblance of the Malayan organism to *Piedraia hortai* is thus apparent Other species of *Piedraia* have been described from South America, these being according to BRUMPT (1936), *P sarmentoi*, *P paraguayo* and *P venezuelensis*, and which are distinguished from *P hortai* by differences either in cultural growth or in the case of *P venezuelensis*, by the formation of four ascospores instead of eight

The Malayan species of *Piedraia* differs from the American *P hortai* in having spores about one-half the size of *P hortai* (*i e* , 4μ or 5μ as compared with 7μ to 12μ), and the name *Piedraia malayi* is therefore proposed

NOTES ON A RECENT CASE OF TRICHOSPOROSIS

The patient was a European, aged 27, who had spent 3 years in Malaya In July, 1949, he noticed numerous black nodules on the hair of the head These occurred only on the front and right side where the hair was brushed most often He used a mucilaginous hair fixative, which was frequently brushed over the affected area

Treatment consisted in cutting away the affected hair, discontinuing the use of the hair fixative, washing the hair with a soap containing 1 per cent mercuric iodide, and applying a lotion containing salicylic acid (2 per cent) and chloretone (5 per cent) There was no recurrence during the subsequent 3 months

SUMMARY

A general account of the hair disease trichosporosis (piedra) is given and a Malayan case described and illustrated This disease has been known to

* Emerson's agar

	G	
Agar	25	
Lemco	4	
Peptone	4	
Sodium chloride	2 5	
Marmite	1	or other yeast extract
Glucose	10	
Distilled water to (adjust to pH — 7 4)	1,000 c c	

occur in South-East Asia for some 30 years and the organism found has so far been regarded as *Trichosporon*.

A step forward, however has been made in showing that the Malayan piedra organism develops ascospores under certain conditions likely to occur naturally or else produced artificially by mucilaginous hair fixatives and it thus conforms with the genus *Piedraia* rather than *Trichosporon*. Because the size of the spores differs from the American species *Piedraia hortai* the name *Piedraia malayi* is proposed for the species here described.

REFERENCES

Brumpt E. (1936) *Précis de Parasitologie* 2019 Paris: Masson et Cie 5th Ed.

Byam, W & Archibald R. G (1923). *The Practic of Medicine in the Tropics* 2375. London: Henry Frowde

Castellani, A. & Chalmers, A. J (1910) *Manual of Tropical Medicine.* 1106 London: Baillière Tindall & Cox.

Faral, P (1939) *Rep Inst med. Res F M S* 72.

Lampe, P H. J (1940). *Geneesk. Tijdschr Ned. Ind.*, 80 1519

Manson Bahr, P H. (1945) *Manson Tropical Diseases.* 660 London Cassell & Co

Moore, M. (1944) *Clinical Tropical Medicine* 653 New York Paul B Hoeber Inc.

Niven J C. (1936). *Malayan Med. Jour* 11 223

Savill, A. (1935) *The Hair and Scalp* 227 London Edward Arnold & Co.

Socchard & Nouten van-Hlong. (1937). *Ann Parasit hum. comp* 15 539

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THE CONCENTRATION OF CERCARIAE OF *SCHISTOSOMA MANSONI* FOR THE PREPARATION OF CERCARIAL ANTIGEN

BY

O D STANDEN
(From the Wellcome Laboratories of Tropical Medicine, London)

The development of experimental schistosomiasis during recent years has provided abundant material for the preparation of schistosome antigens The diagnostic value of the complement fixation technique and intradermal tests is undoubted and the importance of efficient antigenic preparations has not been overlooked The three chief sources of material are infected snails' livers, adult worms and cercariae, all of which provide potent antigens when extracted in a variety of ways In theory, the cercariae should provide the most specific antigens since, unlike the adult worms, they can possess no foreign blood-cells in the gut, and unlike the sporocysts and immature cercariae in snail livers they are not surrounded by molluscan tissues However, as free swimming organisms they are subject to contamination by both organic and inorganic matter, and it is the purpose of this paper to describe a method by which cercariae can easily be concentrated and at the same time freed from the majority of undesirable substances

The chief contaminants of a newly produced cercarial suspension in fresh water are snail faeces, small crustaceans, free living protozoa, rotifers and unicellular algae, though the faeces are by far the greatest in quantity A certain amount of non-living debris is also encountered Attempts have been made to remove snail faeces and other debris by washing centrifuged cercariae with saline and distilled water (OLIVER-GONZALEZ and PRATT, 1944) or straining the suspension through cheese-cloth prior to centrifuging (BOZICEVICH and HOYEM, 1947) The latter found that when using this method faeces were still not eradicated They devised a means of narcotizing the cercariae with 10 per

cent. ethyl alcohol which caused them to sink to the bottom. Since some loss of antigenic potency was encountered as a result of this treatment, the same effect was subsequently obtained by cooling.

The value of the centrifuge in the concentration of suspensions of active living cercariae is open to considerable doubt, and experience in these laboratories has shown this method unsuitable. When spun at 4 000 r.p.m. for 5 minutes, some temporary concentration at the bottom of the tube is obtained, but as soon as the centrifuge has finished turning, many cercariae are found swimming up to the surface again. One or 2 minutes later the suspension is well dispersed or the cercariae may have accumulated near the top. Also, centrifugal treatment of the suspension, whether active or narcotized in some way would serve to carry down the unwanted debris as well. In contrast, it has been observed that snail faeces sink rapidly to the bottom so that they can be separated from the cercariae by causing the latter to concentrate at the surface. A method by which this may be accomplished is described below together with a method of deposition of cercariae upon filter papers and the preservation of the residue. The filter paper technique is a modification of that described by ALVES and BLAIR (1946, 1947) and BLAIR and ROSS (1948). All the cercariae used in the following techniques are those of *S. mansoni* discharged by laboratory bred and experimentally infected *Australorbis glabratus*.

METHOD.

Concentration.

The cercariae producing snails are removed from their aquaria and placed in litre beakers of boiled fresh water at 28° C. and are maintained at this temperature under bright light until a dense cercarial suspension is obtained. About 400 to 500 snails are employed for this purpose, and if placed in the beakers by approximately 9 a.m. the required numbers of cercariae are usually available 1 hour later. Some 100 to 150 snails per litre of water is a convenient number. It may be of interest to note here that a heavy cercarial discharge on 1 day is frequently followed by a resting phase the next day. In practice it has been found of value to divide the positive snail stock into two groups for use on alternate days. The cercarial suspension is now decanted into litre graduated cylinders when the majority of the snail faeces and debris sinks to the bottom. The suspension is now decanted and filtered through mosquito netting into a cylindrical separating funnel of suitable capacity (Fig.). Netting of 23/24 mesh is a suitable type and permits the free passage of cercariae while retaining floating debris and crustaceans. To prevent mechanical destruction of the relatively delicate cercariae, the stem of the filter funnel is extended almost to the bottom of the separating funnel. The latter is inserted into the inverted top half of a Winchester quart bottle which when filled with ice cold water provides a cooling jacket. The top of the funnel is brightly illuminated from the sides. The cercariae are thereby induced to concentrate in the top 2 or 3

inches of water in response to their negative geotropic, positive phototropic and thermotropic characters Any remaining faecal sediment is left at the bottom Newly discharged cercariae will concentrate in this manner in a very few minutes and the lower and relatively cercariae-free layer is then run off Further quantities of cercarial suspension may now be added and the cercariae re-concentrated in the same way The final 200 to 250 c c of suspension is usually very dense but contains some protozoa, algae, rotifers, etc It is not possible to eliminate these entirely but their numbers can be reduced To do this, the suspension still in the separating funnel is diluted to the capacity of the funnel with sterile distilled water at 28° C, and separation is again made

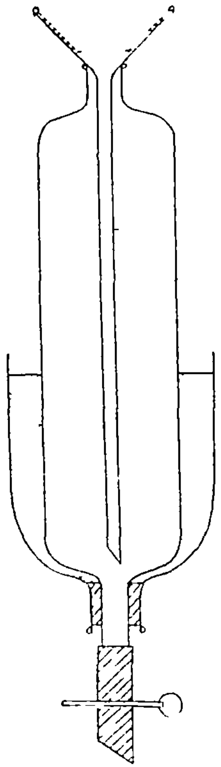

FIG —Apparatus employed for the concentration of cercarial suspensions

This process of dilution may be repeated two or three times provided that the cercariae have not been allowed to expend their energy for more than 2 or 3 hours previously, since after this time a large proportion may lack the energy for rapid surface concentration This washing serves to dilute the contaminating organisms The final separated concentrate of some 200 to 250 c c is run off into a graduated cylinder from which sampling counts are made and the approximate number of cercariae estimated by volume

Filtration

The counted cercariae are now filtered. Several methods have been investigated but the most successful apparatus is a Hirsch funnel, of 22 mm. plate and Whatman No. 54 filter paper. Prior to filtration, treatment of the filter paper with a very dilute suspension of kieselguhr in water serves to prevent escape of cercariae which tend to slip underneath and are thereby lost. If the algae and other foreign organisms have been removed the cercariae appear as a pale creamy deposit. The filter paper is now removed and, whilst still damp is rolled into a loose cylinder and placed in a bijou bottle with the number of cercariae and the date noted on the outside. The open bottle is placed in a desiccator and the paper dried *in vacuo* over phosphorus pentoxide. When the paper is thoroughly dry the bottle is screwed down and placed in cold store.

The object of concentration and rapid filtration is to ensure that the majority of the cercariae are still alive by the time the last drop of water passes through the filter and that they are, in fact, killed by desiccation. It is considered that any water passing over dead material would tend to leach out the antigenic substances and this, of course, also precludes any additional concentration upon an already charged paper. Provided sufficient positive snails are available 22 mm. filter papers of 200 000 to 250 000 cercaria-value can be obtained quite easily. The papers should be of as small a size as possible since a large bulk of filter paper requires too great a volume of extracting medium for the preparation of highly concentrated antigens.

SUMMARY

1. A method of concentrating cercariae of *Schistosoma mansoni* is described. The negative geotropic, the positive phototropic and thermotropic characteristics of the cercariae are utilized to concentrate them in the upper layers of water whilst snail faeces remain as sediment. Micro-organisms [illegible]re removed as far as possible by dilution of their numbers.

2. Deposition of large numbers of cercariae upon a small filter paper [illegible] described. The need for high cercarial concentration [illegible]nd speed of filtr[illegible]tion [illegible] emphasised as a means of preventing the death of the cercariae before completion of filtrat[illegible]on and c[illegible]nsequent leaching out of [illegible]nt[illegible]genic substances.

REFERENCES

ALVES, W. & BLAIR, D. M. (1946). *Lancet*, 1, 558.
——— ——— (1947). *S. Afr. med. J.*, 21, 35.
BLAIR, D. M. & ROSS, W. F. (1949). *Ann. trop. Med. Parasit.*, 43, 46.
BOZICEVICH, J. & HOYEM, H. M. (1947). *Nat. Inst. Hlth Bull.*, 189.
OLIVER-GONZALEZ, J. & PRATT, C. K. (1944). *Puerto Rico J. publ. Hlth trop. Med.*, 20, [illegible]

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A PRELIMINARY TRIAL OF PALUDRINE SINGLE-DOSE THERAPY IN COLUMBIA *

BY

HERNANDO REY,
División de Malariología, Co-operative Inter-American Public Health Service, Colombian Ministry of Health,

AND

JULIAN DE ZULUETA,
Instituto de Enfermedades Tropicales Roberto Franco, Colombian Ministry of Health

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One of the main problems of malaria control in the tropics is the treatment of malaria attacks in rural areas where the population will not, or cannot, afford to follow a full course of treatment The Report of the Second Session of the Expert Committee on Malaria of the W H O (1948) calls attention to the advantages of clinical control of malaria in such areas by means of a single dose of paludrine Extensive work has been carried out recently in India showing that a single dose of paludrine mg 300 is sufficient in the great majority of cases to control an attack of vivax or falciparum malaria AFRIDI (1947), in his review of therapeutic trials on paludrine, carried out under the direction of the Malaria Institute of India, suggests that a single dose of paludrine mg 300

* The authors are indebted to the assistance of Sr ANTONIO ORDUZ, of the Roberto Franco Institute, and the staff of the Villavicencio Hospital, for the follow up of the cases reported here

is the most desirable course of treatment for malaria in that country both in the dispensaries and the hospitals.

In the present paper are summarized the results of 15 cases of malaria treated with a single dose of paludrine mg 300 Although the number of cases studied is small, a report seems warranted in view of the scarcity of published records on the effect of paludrine on the South American strains of malaria plasmodia. The patients came from rural areas to receive hospital or dispensary treatment in Villavicencio. All had a history of previous malaria attacks and none of them had received any anti malarial drug in the days preceding the administration of paludrine The drug was given by mouth in all cases and special care was taken to make sure that vomiting did not occur after its administration. No toxic after-effects were noticed in any of the cases although two of them were 3-year-old children to whom we had nevertheless given the same dose. The cases were followed up for a period of 15 days after the treatment daily blood films were taken in addition to the ordinary medical care. Paludrine tablets mg 300 coloured red, produced by Imperial Chemical (Pharmaceuticals), Ltd. were used in these trials. The results of the clinical and haematological findings are summarized in the table which follows.

As can be seen, in all cases except one, the fever was controlled within the first 3 days of the administration of the paludrine tablet and the trophozoites disappeared from the peripheral blood within the same period. The average duration of the fever in the 14 cases controlled by paludrine in the dose stated was 1·8 days, and the average duration of the trophozoite parasitaemia was 1·4 days. It can also be observed that the only case in which the treatment failed to control the clinical attack (No. 14) was that with the highest parasitaemia, and which ran the most severe clinical course. In No. 7 however although the fever was at first controlled and parasites disappeared from the blood, a parasitic and clinical relapse took place within the 15-day period of observation and this was a case with mild symptoms and a low parasitaemia.

Of the nine falciparum cases, including No. 15 a mixed infection of *Plasmodium vivax* and *P falciparum*, the gametocytes were cleared out of the peripheral blood in only two cases. There was a marked tendency for the gametocytes of this species to remain in the blood or to appear in it after the administration of paludrine. The vivax gametocytes, however showed a different behaviour In our seven cases of vivax infection (including again No. 15), the gametocytes disappeared from the blood within the first 7 days after treatment

The results of the 15 cases summarized in this note agree in general with those obtained in India (AFRIDI, 1947) and in Brazil (PINTO, 1947). This preliminary trial, although conducted on a small scale suggests that this simple treatment is advisable for the control of malaria attacks in the rural areas of this country where a complete course of treatment is seldom practicable and where

TABLE—EFFECT OF PALUDRINE MG 300 ON CLINICAL COURSE AND PARASITAEMIA OF 15 UNSELECTED CASES OF MALARIA.

Serial number	Age, years	Sex.	Type of clinical attack	Spleen enlargement.	Parasite species	Parasites per c mm	Duration of fever, days	Duration of parasitaemia, in days		Gamet appearing in peripheral blood, day	Parasitic relapse troph, day	Clinical relapse day
								Troph	Gamet			
1	15	F	Moderate	PDI	*P vivax* T & G	16,300	2	2	3	—	—	—
2	3	M	"	I	*P falcip* T & G	15,700	2	1½	15	—	—	—
3	40	M	Mild	O	*P falcip* T	500	1	1	—	14th	—	—
4	15	M	"	I	*P falcip* T & G	800	1	1	15	—	—	—
5	30	M	Moderate	O	*P falcip* T	8,000	3	1	—	—	—	—
6	21	M	Mild	PDI	*P vivax* T & G	4,700	1	1	1½	—	—	—
7	16	M	"	I	*P falcip* T & G	5,300	3	1	5	—	11th	14th
8	8	M	"	I	*P vivax* T & G	5,700	1½	1½	1½	—	—	—
9	3	F	Moderate	I	*P falcip* T & G	12,400	1½	1	15	—	—	—
10	21	M	"	O	*P vivax* T & G	6,800	2	2	4	—	—	—
11	22	M	Severe	I	*P falcip* T	121,600	2	1	—	6th	—	—
12	18	M	Moderate	II	*P vivax* T & G	2,800	2½	3	7	—	—	—
13	22	M	Severe	I	*P vivax* T	34,300	1½	1	—	—	—	—
14	18	M	"	II	*P falcip* T & G	144,000	(*)	(*)	(*)	(*)	(*)	(*)
15	12	M	Moderate	I	*P vivax* T & G *P falcip* T	14,100	1	1	1 *vivax*	2nd *falcip*	—	—

most patients coming under medical attention have a previous history of malaria and have thus presumably a certain degree of immunity. COVELL, NICOL, SHUTE and MARYON (1949) have shown recently that in cases in which immunity has not been built up very different results are to be expected from the use of paludrine.

REFERENCES.

AFRIDI, M. K. (1947). *Indian J. Malariology* 1, 347.
COVELL, G., NICOL, W. D., SHUTE, P. G. & MARYON, M. (1949). *Trans. R. Soc. trop. Med. Hyg.* 42, 465.
PINTO, G. DE S. (1947). Reprinted from *Folha Med.*, 5th August.
WORLD HEALTH ORGANIZATION. INTERIM COMMISSION. (1948). Expert Committee on Malaria. Report of Second Session. Washington, D.C., May 19th to 25th.

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OSCILLOMETRIC STUDIES IN NEURAL LEPROSY *

BY

JAMES BARNETSON, M D (PRETORIA), M B, CH B (EDINBURGH), D T M & H (WITWATERSRAND)

Institute for Pathology, University of Pretoria

Investigations of peripheral circulatory changes in neural leprosy have seldom been recorded LEITNER (1938) injected radio-opaque material into the anterior tibial artery of a female with leprous multilations and "mal perforant" of the foot and demonstrated richness and patency of arterial network even as far as the ulcer FAGET and MAYORAL (1944), as a result of arteriographic studies in all types of leprosy, stated that arterial supply in extremities is not materially disturbed in neural leprosy

Various observers (TEDESCO and MAZZOLENIS, 1925, KOMATSU, 1937, RIVELLONI, 1938), by means of the capillaroscope observed various anomalies of skin capillaries in leprosy FITE (1941), investigating blood-vessels in various types of leprosy, found only one case with vascular lesions out of 11 neural cases examined The lesion was tuberculoid thrombophlebitis in a subcutaneous vein Several authors have noted hyperaemia and feeling of warmth in affected limbs after sympathectomy for leprous ulcerations (OSAWA and NOJIMA, 1927, CRUZ, ABUEL and SAMSON, 1931, BLACK, 1933, MARTY, 1938, KIRKALDY-WILLIS, 1945)

In the present investigation the peripheral arteries of 37 neural lepers were examined by means of the oscillometer This instrument is used to investigate changes in volume of pulsation in limbs by measuring the amount

* I am indebted to Dr A R DAVISON, Superintendent of the Westfort Leper Institution, Pretoria, for generous facilities placed at my disposal, and to Dr H J F WOOD, of the same Institution, for help during the performance of the tests

of pulsation transmitted to an air filled cuff applied to the limb under examination. It indicates the total pulsation of all vessels encompassed by the cuff The instrument is applied like a sphygmomanometer and inflated. The indicating needle moves with each heartbeat and, as extent of movement depend largely on pressure within the cuff readings are taken at various pressures in order to record maximal excursions.

The oscillometer is valuable for diagnosis of obliterative lesions of arteries and arterioles of limbs but cannot help in differentiating various causes of reduced flow

Interpretation of readings is rendered difficult by wide variations in normal subjects and in the same subject in different circumstances. SAMUELS (1941) gives the normal range in regions of wrist and ankle as between 1 and 10. Several workers (ATLAS, 1939 and 1940 RINZLER, TRAVELL and CIVIN 1944) compared readings at wrist with those at ankle. Using latter as numerator and former as denominator the "oscillometric index" is obtained. This is usually greater than unity as arterio-sclerotic disease is more frequent and more advanced in lower than in upper extremities. The lower limit of normal is usually given as 0·75

In the present investigations a Collen's sphygmooscillometer was used. The scale units correspond to mm. of mercury as in a sphygmomanometer

MATERIAL INVESTIGATED.

Thirty-seven adult native neural lepers were examined, 20 classified radiologically or clinically as early and 17 advanced. Patients were semi recumbent in a well-ventilated room and readings were taken at both wrists and both ankles with cuff inflated to following pressures 40, 60 80 100 120 140, 160 180 200 and 220 mm. of mercury Maximal pulsations only were recorded and results were as follows

ANALYSIS OF RESULTS.

In early cases the lowest reading at wrists was 1 5 and at ankles 2 5. The average reading t wrists was 2 7 and at ankles 4 All oscillometric indices were either unity or over except case 9776, in which it was 0·8 on both sides. The average oscillometric index was 1 5 right side and 1·6 left side.

In advanced cases the lowest reading at wrists was 1 and at ankles 2. All oscillometric indices were either unity or over The average at wrists was 2 1 right side and 2·0 left side, and at ankles 3 3 right side and 3·4 left side The average oscillometric index was 1 7 right side and 1 8 left side. Although average results in this group were slightly lower than in the early group, this was not considered significant as (a) all readings were within normal limits (b) the average oscillometric index in the advanced group was not lower than in the early group.

EARLY CASES

Patient number	Right wrist.	Right ankle	Oscillometric index	Left wrist	Left ankle	Oscillometric index.
9184	2 0	5 0	2 5	2 0	5 0	2 5
9291	3 0	3 5	1 2	3 0	3 5	1 2
9889	2 5	4 0	1 6	2 5	4 0	1 6
9816	2 5	2 5	1 0	1 5	3 0	2 0
9776	3 0	2 5	0 8	3 0	2 5	0 8
9865	2 5	2 5	1 0	2 5	2 5	1 0
9870	2 5	5 0	2 0	2 5	5 0	2 0
9906	2 5	3 0	1 2	2 5	2 5	1 0
9984	2 0	5 0	2 5	2 5	5 0	2 0
9886	1 5	4 0	2 7	1 5	3 5	2 3
9911	3 0	3 5	1 2	3 0	3 5	1 2
9913	3 5	4 0	1 1	3 5	4 0	1 1
9916	3 0	4 5	1 5	3 0	4 5	1 5
9948	4 0	5 0	1 3	4 0	5 0	1 3
9969	2 5	2 5	1 0	2 5	3 0	1 2
9984	2 0	5 0	2 5	2 0	5 0	2 5
10015	3 0	5 5	1 8	3 0	5 5	1 8
10016	3 5	5 0	1 4	3 5	5 0	1 4
10022	2 5	2 5	1 0	2 5	2 5	1 0
10031	3 0	4 5	1 5	2 5	4 5	1 8
Average	2 7	4 0	1 5	2 7	4 0	1 6

ADVANCED CASES.

Patient number	Right wrist.	Right ankle	Oscillometric index	Left wrist.	Left ankle	Oscillometric index.
6388	3 0	3 5	1 2	3 5	3 5	1 0
6729	2 5	6 0	2 4	2 5	6 0	2 4
7101	1 5	3 5	2 3	1 5	4 0	2 7
7694	2 0	4 5	2 3	2 0	4 5	2 3
8528	2 0	3 5	1 8	2 0	3 5	1 8
8832	1 5	2 0	1 3	1 5	2 0	1 3
8890	2 5	3 0	1 2	2 0	3 0	1 5
9329	1 5	3 0	2 0	2 0	3 0	1 5
9563	2 5	4 5	1 8	2 0	4 5	2 3
9732	2 0	3 0	1 5	2 0	3 0	1 5
9762	2 5	2 5	1 0	2 5	2 5	1 0
9739	1 5	4 0	2 7	1 5	4 5	3 0
9762	2 5	2 5	1 0	2 5	2 5	1 0
9821	1 5	3 5	2 3	1 5	3 5	2 3
9845	2 5	3 0	1 2	2 0	2 0	1 0
9859	2 0	2 0	1 0	2 0	2 0	1 0
9901	1 5	2 5	1 7	1 0	3 0	3 0
Average	2 1	3 3	1 7	2 0	3 4	1 8

CONCLUSION

There is no organic occlusion of arteries and larger arterioles of wrist and ankle regions in neural leprosy These findings accord with arteriographic and histological findings.

REFERENCES

ATLAS L. N (1939). *Arch int Med.*, **63** 1158.
—— (1940) *Ibid.*, **66** 155.
BLACK, K. (1933) *Malayan Med. J* **8** 60 (Abst. *Trop Dis Bull.*, **30** 553.)
CRUZ, M. C., AGUIAR, J L. & SAMSON J G (1931). *J P I Med Ass* 11 474 (Abst *Trop. Dis. Bull.*, **29** 549)
FAGET G H & MAYORAL, A. (1944) *Radiology* **42** 1
FITE, G L. (1941) *Int J Leprosy*, **9** 193.
KIRKALDY WILLIS, W H. (1945). *E Afr med. J* **22**, 88.
KOWATARI S. (1937). *Jap J Derm Urol.* 41 13. (Abst. *Int. J Leprosy* **5** 542.)
LATTNER, A. J (1938) *Int J Leprosy* **6**, 471
MARTY J (1938) *Bull. Soc Path. exot* **31** 199 (Abst. *Trop Dis. Bull.*, **35** 556)
OKAWA, Y & NOJIMA, T (1937). *Dtsch. med. Wschr.*, **64** 65
RINZLER, S H., TRAVELL, J & CUTOX A. (1944). *Arch. int. Med* **73** 241
RIVELLONI, G (1938) *G ital Derm. Sif* **79** 281
SAMUELS, S. S (1942) *J Lab clin. Med.*, **27** 19
TEDESCO, P A. & MAZZOLENTI, U (1925). *G Clin. med.*, **6** 175 (Abst *Trop Dis. Bull.*, **22** 579)

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SKIN TEMPERATURE STUDIES IN NEURAL LEPROSY *

BY

JAMES BARNETSON, M D (PRETORIA), M B , CH B (EDINBURGH), D T M & H (WITWATERSRAND)
Institute for Pathology, University of Pretoria

Skin temperature determinations are commonly used in studying peripheral circulatory anomalies Skin temperature is influenced by many factors, including environmental temperature, posture and basal metabolism Experimental conditions must therefore be standardized and maintained The present study is concerned with reaction to the following imposed variations in vasomotor activity

(1) Local warming and cooling of feet
(2) Placing a hand and forearm in cold or hot water to elicit reflex vasomotor changes in other limbs

Response to Local Warming and Cooling of Feet

Initial temperatures were recorded after patients had been recumbent for at least 30 minutes with limbs exposed in a well-ventilated room with fairly constant temperature A skin temperature thermometer of light-beam galvanometer type was used and readings made on dorsum of great toe just proximal to nail bed When great toe was missing a neighbouring digit or distal end of the stump was used

In the first series one foot was immersed in water at 110° to 115° F for 20 minutes, withdrawn, dried, and the temperature estimated every 4 minutes for 1 hour In the second series the procedure was similar but the foot was immersed in ice-water for 20 minutes

Seventeen neural lepers, all adult native males, were so investigated, eight classified radiologically and clinically as early cases and nine as advanced

The results are given in the Tables which follow

* I am greatly indebted to Dr A R DAVISON, Superintendent of the Westfort Leper Institution, Pretoria, for generous facilities placed at my disposal, and to Dr H J F. WOOD, of the same Institution, for his help during the performance of the investigations.

RESPONSES TO LOCAL WARMING OF FEET

Patient.	Initial temperature.	Just after warming	After 60 minutes.
Early Neural Cases.			
9184	65	90	73
9680	73	91	73
9016	77	93	85
9935	86	89	85
9943	73	90	71
9984	77	90	85
10022	67	90	70
10031	77	92	87
Advanced Neural Cases.			
5881	68	89	72
6388	84	94	84
6729	80	90	86
7018	84	91	82
684	91	94	89
8328	81	92	82
9184	69	89	72
9329	89	94	84
9901	80	89	84

RESPONSES TO LOCAL COOLING OF FEET

Patient	Initial temperature	Just after cooling	After 60 minutes
Early Neural Cases			
9184	72	Below 50	74
9689	78	50	77
9916	73	60	62
9935	88	65	64
9948	76	Below 50	73
10022	73	Below 50	71
10031	81	65	62
9984	77	59	74
Advanced Neural Cases			
5881	72	Below 50	70
6388	84	60	64
6729	78	58	64
7018	79	61	60
7094	61	68	64
8528	79	56	74
9184	69	Below 56	74
9329	88	58	66
9901	75	57	81

In the warming experiments a gradual return of temperature after removal from the water was seen. In the cooling series there was satisfactory rate of rise of temperature and in several instances it exceeded that initially recorded, indicating vasodilatation. These results corresponded closely to five normal controls and showed that in the feet of neural lepers, dilatability of cutaneous and subcutaneous bloodvessels in response to local stimulation was unimpaired.

Reflex Vasodilatation. When a limb is kept immersed in water at 110° to 115° F vasodilatation will normally take place in non-immersed limbs after 7 to 20 minutes (RICHARDS, 1948). The mechanism of this reflex dilatation is still under discussion (GIBBON and LANDIS, 1932 PICKERING, 1937 DUTHIE and MACKAY 1940 RICHARDS 1946 ALLEN BARKER and HINES, 1946). Warm blood returning from the heated limb probably forms the afferent pathway of the reflex arc and sympathetic nerve fibres to limbs the efferent.

After 30 minutes recumbency with limbs exposed in well-ventilated room, initial temperatures were recorded on dorsum of great toe just proximal to nail-bed and a corresponding position on the thumb. In absence of these digits neighbouring digits or the end of the stump were used. The other hand and forearm were then immersed in ice-water for 30 minutes and temperatures of the non-immersed hand and foot taken. Immersed hand and forearm were then transferred to water kept between 110° and 115° F until reflex vasodilatation was obtained or otherwise for at least 1 hour

Thirty four adult native males were investigated, 20 classified radiologically and clinically as early and 14 as advanced neural lepers. Although those with clinical or X ray evidence of sepsis were excluded it was noted that when the initial temperature was 80° F or over response was poor It was concluded that these high initial temperatures were due to undetected sepsis, and they were also discarded, leaving 18 early and 12 advanced cases.

The following tables show the temperature range (lowest and highest recorded temperatures), the temperature rise and the interval in minutes between time of immersion in hot water and significant vasodilatation or attainment of maximum temperature

In all normal controls vasodilatation began within 20 minutes and was complete in 30.

The results showed failure of reflex-dilatation in non-immersed limbs after immersing hand and forearm in hot water The degree of failure ran parallel to that of neurotrophic change (particularly bone atrophy), but this correlation only held good for groups of leper patients and not for individuals. Thus in early cases vasodilatation was good but there were individual exceptions (9 184 thumb 9,865 toe, 9,911 thumb and toe, 9,913 thumb). Similarly in advanced cases reflex vasodilatation was poor but with exception (5,881 thumb and toe 7,018, 7 101 and 7 694 thumb 9 490 toe)

The degree of diminution of reflex vasodilatation corresponded roughly also to the degree of anaesthesia of limbs, but again with exception. Some completely anaesthetic extremities showed fairly good reflex responses and some partially anaesthetic hands and feet greatly diminished reflex reactions.

REFLEX VASOMOTOR EXPERIMENTS

Patient	Thumb			Big toe		
	Range	Rise	Interval	Range	Rise	Interval
Early Neural Cases						
9184	75–81	6	42	72–90	18	36
9290	77–91	14	18	70–89	19	45
9689	77–90	13	24	76–90	14	30
9776	65–93	28	24	63–75	12	33
9816	65–90	25	21	64–83	19	36
9825	71–90	19	33	68–89	21	60
9865	74–89	15	27	70–73	3	60
9870	70–90	20	30	79–89	10	45
9886	76–89	13	15	71–82	11	39
9906	75–90	15	24	70–86	16	39
9911	75–78	3	45	71–74	3	45
9913	74–80	6	48	70–88	18	45
9916	74–90	16	24	75–90	15	33
9948	—	—	—	80–91	11	24
9969	—	—	—	72–91	19	21
10016	—	—	—	78–90	12	51
10022	—	—	—	76–89	13	27
10031	79–90	11	24	68–90	22	27
Advanced Neural Cases						
5881	62–74	12	27	62–79	17	27
6274	70–76	6	48	66–73	7	51
6388	74–75	1	39	—	—	—
6729	69–75	6	48	—	—	—
7018	65–76	11	42	—	—	—
7101	73–86	13	45	77–84	7	36
7694	74–84	10	42	—	—	—
8528	69–77	8	39	78–85	7	39
8832	74–80	6	42	79–85	6	48
9490	80–86	6	39	62–72	10	45
9732	78–88	10	36	—	—	—
9901	75–82	7	45	70–79	9	54

	Average Rise of Temperature		*Extremes*	
	Thumb	Big toe.	Thumb	Big toe
Early cases	14·6	14·2	3–28	3–22
Advanced cases	8	9	1–13	6–17
Normal controls (5)	22·6	20·6	19–29	16–25

All patients, even when anaesthesia in skin of forearm and hand was complete, complained of pain during immersion in ice water.

Conclusions.

Dilatability of peripheral vessels in neural lepers in response to local stimulation is not impaired.

There is failure of reflex vasodilatation corresponding roughly to degree of neurotrophic bone change and anaesthesia.

The cause of such failure is evidently destruction of vasomotor fibres in peripheral nerves by leprous neuritis. In early cases few fibres are destroyed and responses are adequate. As destruction of nerve fibres progresses there is increasing failure of reflex vasodilatation.

Anomalous results can be accounted for as follows. In advanced cases surviving vasomotor fibres may allow some reflex vasodilatation whilst occasionally in early cases vasomotor fibres may be affected first. "Neurotrophic" changes in leprosy of course depend on more factors than nerve damage, such as infection, and repeated trauma in hyposensitive tissues.

REFERENCES

Allen, E. V., Barker, N. W. & Hines, E. A. (1946). *Peripheral Vascular Diseases.* 1st Ed. 106. Philadelphia: W. B. Saunders Co.

Duthie, J. J. R. & Mackay, R. M. I. (1940). *Brain*, 63, 295.

Gibson, J. H. & Landis, E. M. (1932). *J. clin. Invest.*, 11, 1019.

Richards, R. L. (1946). *The Peripheral Circulation in Health and Disease.* 1st Ed. 44 and 46. Edinburgh: E. & S. Livingstone Ltd.

This article is abstracted from a portion of a thesis accepted by the University of Pretoria in fulfilment of the requirements for the degree of M.D.

TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. 43. No. 5. March, 1950.

THE GROWTH OF A MAGGOT ON STERILE BLOOD

BY

C. GARRETT-JONES, B.A. (Oxon.), M.Sc.
London School of Hygiene and Tropical Medicine.

Insects which are haematophagous in the growing stage often present particular difficulties in work on nutrition because they will neither feed readily on free liquids nor grow under aseptic conditions. WELLINGTON (1949) has drawn attention to similar difficulties with phytophagous species.

I have recently had a partial success in this direction with the Congo floor maggot (*Auchmeromyia luteola*). This species is the only dipterous parasite specific to man, the larva being a free living but obligatory bloodsucker while the fly lives on faeces and sweets. I have cultivated it in the laboratory for 3 years (by a method to be described fully elsewhere), normally rearing the maggots on a human host. In seeking alternative techniques, however, it was found that they can be grown at least in part on free sterile blood.

This was done by pipetting a few drops of the prepared blood on to a small disc of filter paper in a petri dish. The maggots were placed near the blood and incubated at 23° C. The cuticle of the maggot is exceptionally hydrophilic (PAL, in press) and as soon as it touched the liquid the whole body became enveloped in a film of blood. Some deaths may have been due to the omission to wash the maggots after they had fed.

First, 15 newly hatched maggots were offered citrated rabbit blood. Most imbibed some of it but few gorged, and after the second such meal deaths began to occur. By the 12th day there was only one survivor, which, however, continued to feed and grow until the 23rd day, when this test was abandoned. No further attempt was made to rear the larva throughout on free blood.

A batch of maggots, fed on man until the second moult, was then transferred to a diet of citrated rabbit blood. Five out of 12 were still feeding on the 14th day, and two of these pupated and produced adults. In another batch transferred from the natural diet, three out of six pupated after taking five meals of defibrinated rabbit blood and one of citrated. Similar results were obtained using oxalated horse blood and again with heparinized human blood. All the larvae weighed 10 to 20 mg. at the time of transfer, and the survivors reached 100 mg. or more before they pupated.

The readiness of the floor maggot to imbibe free liquid may be connected with the form of its mouthparts. The few dipterous larvae which suck vertebrate blood (comprising, besides *Auchmeromyia*, members of three or four genera parasitic on young birds in the nest) differ from almost all blood-sucking arthropods in that they lack any tubular proboscis or piercing stylets. *Auchmeromyia* possesses only the blunt mouth hooks and minute maxillary

teeth characteristic of cyclorrhaphous larvae. After scraping a wound in the host's skin it closely applies the soft hydrophilic cuticle and can then suck the blood directly into the pharynx, enlarging the wound from time to time by working the mouth hooks back and forth.

This structure and mode of sucking show the affinity of the Congo floor maggot to other blowflies. The larvae of *Lucilia* and *Calliphora* can likewise be fed on free blood (Hobson, 1933). The same is true, however, of at least some insects with a piercing proboscis, such as mosquitoes (Russell, 1931; Mattingly, 1946).

My results suggest interesting physiological problems. It seems not to have been previously noted that the handful of diptera I have referred to are perhaps the only arthropods which live upon vertebrate blood in the growing but not in the reproductive stage. One wonders whether they possess symbiotic micro-organisms to supply them with growth factors in which their diet is deficient, but which are thought to be required by all insects (Trager, 1947). Wigglesworth (1929) remarked the fact that such symbionts are absent in those insects which suck blood only as adults, but present in those which take no other food at any stage of their life cycle. They seem to be more widely required for growth than for ovarian development, though in *Pediculus* they are necessary for both (Wigglesworth, 1939) and not all mosquitoes can produce viable eggs on a diet of sterile blood (Mattingly, 1946).

I have found no record of any search for micro-organisms in a bloodsucking maggot. The excreta of *Auchmeromyia* larvae, like those of *Lucilia* and *Calliphora*, are rich in ammonia, strongly indicating that bacterial decomposition occurs in the gut, although in those genera much of it comes from the tissues themselves (Wigglesworth, 1939).

While in some insects a symbiont is transmitted to the interior of the egg, in others the eggshell is contaminated so that the larva becomes infected as it hatches (Trager, 1947). Hobson (1933) found that blowfly larvae would not grow on sterile blood when the living eggs had been sterilized, whereas larvae from unsterilized eggs did grow slowly on the same diet. In view of the feeding habits of *Auchmeromyia*, it seems more probable that in this genus the egg is contaminated by the female than that the larva relies upon picking up bacteria from its habitat or from the skin of the host. However this may be, the floor maggot, which is easily reared in the laboratory, may prove to be a convenient subject for studying problems of the growth and metabolism of larvae fed upon fluids of known composition.

REFERENCES

Hobson, R. P. (1933). *Biochem. J.* 27, 1899.
Mattingly, P. F. (1946). *Nature* 158, 751.
Pal, Rajendar. *Bull. ent. Res.* (In press.)
Russell, P. F. (1931). *Amer. J. trop. Med.*, 11, 355.
Trager, W. (1947). *Biol. Rev.* 22, 148.
Wellington, E. F. (1949). *Nature* 163, 574.
Wigglesworth, V. B. (1929). *Parasitol.* 21, 288.
—— (1939). *The Principles of Insect Physiology.* London: Methuen & Co., Ltd.

TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE
Vol 43 No 5 March, 1950

CORRESPONDENCE.

FUMIGANT AND REPELLENT EFFECTS OF BHC (GAMMEXANE) AND DDT UPON ANOPHELES

To the Editor, TRANSACTIONS *of the Royal Society of Tropical Medicine and Hygiene*

SIR,

Two field workers, GEBERT (1948), and more recently GABALDON (1949), both writing in this journal, have suspected a repellent effect of DDT upon anopheles Their suspicions are supported by recent observations made by Mr J A REID, Entomologist of this Institute, whose findings, summarized below, may perhaps be of interest to other workers in this field A fuller account will appear later

Observations made at this Institute, in the field on *Anopheles maculatus** and in the laboratory on *A vagus*,† suggested that BHC was acting as a repellent at a distance Experiments confirmed this, and in addition seemed to show a similar effect with DDT, though much less pronounced The experiments also showed a marked fumigant action by both insecticides , this is well known with BHC , but not with DDT Indeed, several workers (DUSTAN *et al* , 1947, HOFFMAN *et al* , 1949) have failed to find any fumigant effect in laboratory experiments with this insecticide

The experimental method was to count the number of *A vagus* resting by day on one gauze side of a small cage containing about 50 adults of this species, male and female, and then to bring up a plywood panel treated with the substance under test, to about 1 inch from the side of the cage The panel was held motionless there for 15 minutes and the mosquitoes then recounted The same thing was done simultaneously with a control cage and untreated panel Counts were made in this way on three sides of each cage, and the sums of these formed one test After testing, each cage was kept for 24 hours and the number of dead mosquitoes then counted The results are summarized in the table

EFFECT OF PLACING TREATED NILS FOR SHORT PERIODS ABOUT 1 INCH FROM RESTING ADULTS OF *Anopheles* cages.

Treatment.	Number tests.	Number mosquitoes resting on side of cage. Sum of tests. At start S.	At finish F	F/S	Mortality 4 hours after exposure. Number	Per cent.
DDT	5	93	73	0.78	110/246	45
BHC	2	43	15	0.35	97/114	81
Citronella	1	30	8	0.27	4/30	7
Control	9	239	43	1.0	10/326	6

DDT 200 mg. per sq. ft. applied as wettable powder (4 tests), and 400 mg. per sq. ft. as benzene solution (1 test). BHC 40 mg. gamma isomer per sq. ft., wettable powder and 80 mg benzene solution. Citronella 0.3 c.c. applied in drops to the panel, area 0·25 sq. ft. All cages contained about 50 mosquitoes, but only proportion of these rested on the sides where counts were made so that the figures in columns S and F are considerably less than the totals employed, which appear in the first column of mortality e.g. 246 114, etc

The table shows that both BHC and DDT caused considerable mortality presumably due to a fumigant effect, because in other experiments, where the possibility of solid particles coming in contact with the mosquitoes appeared to be effectively excluded, there was again a high mortality. There also appears to be a true repellent effect, which with BHC is comparable with that produced by citronella, though the latter causes no subsequent mortality. The probabilities that the ratios F/S for BHC and DDT could be due to chance are less than one in 1000 and less than one in 50 respectively.

Mr Reid's observations were made in the course of other work and could not be pursued further. They seem convincing, but he feels they need confirmation.

I am, etc.,

JOHN W. FIELD.

Institute for Medical Research,
Kuala Lumpur
Malaya.
December 1949

REFERENCES.

…AM, G. G. ARMSTRONG, T. & PUTNAM, W. L. (1947). *Canad. Ent.* 79, 45.
…LDON, A. (1949). *Trans. R. Soc. trop. Med. Hyg.* 43, 113.
…T, S. (1948). *Ibid.*, 42, 295
…AM, R. A. & LINDQUIST, A. W. (1949). *J. econ. Ent.*, 42, 436.
…, J. A. *Bull. ent. Res.* In the press.
…URTON, R. H. & REID, J. A. *Nature*. In the press.

CORRESPONDENCE

FILARIA IN THE SUDAN

SIR,

Concerning Dr BLOSS' remarks on a few points in my paper on Filaria, which were contained in his letter to these TRANSACTIONS (1949, **43**, 236)

(*a*) *Loa loa* It is quite possible that in certain seasons *Chrysops* are more commonly seen My observations, which gave special attention to chrysops' habits over 7 years, were definitely that they could seldom be found except on cattle They would not be described as "vicious" relative to tsetse fly by the natives who were questioned concerning them, and they knew the fly well having different names for two different species of them

Great variation is noticed in the numbers of certain species of biting flies in different years I have observed this especially with *Hippocentrum* Dr BLOSS' observation (and mine connected with *C silacea*) suggests that the chrysops population does fluctuate a certain amount

All the European patients I knew were well acquainted with *Chrysops*, in which they took a special interest

The evidence remains strong that there is a vector of *Loa loa* other than *Chrysops*

(*b*) *O volvulus* The map attached to my paper was on too small a scale to mark the little patch of uninhabited area referred to A river containing *Simulium damnosum* runs through it

The mark, "Sue I," should incidentally have been north-west, instead of south-east, of Li Rangu

The ophthalmic surgeon, Dr R. MCKELVIE, examined a great number of eye cases at Mvolo with the ophthalmoscope and demonstrated a long series to me, and some at Wau A few of these had occipital nodules I cannot recall any nearer the eye than the occiput RIDLEY's (1945) observation is indeed worthy of special note Dr BLOSS agrees with me that "the intensity of infection may be something to do with the incidence of eye lesions"

In the area "Sué II," where Dr KIRK and I did a survey, no ophthalmoscopic examinations were done but the interesting point was that there were so few eye cases of any kind, filarial or other, in an area showing 77 per cent of positive skins

Dr BLOSS has recently made the interesting discovery of the existence of a new site of *O volvulus* on the Upper Nile-Ethiopian border My paper had already gone to press before this piece of information could be included

There are undoubtedly other streams and sites in the Southern Sudan, including even the area referred to in my paper, likely to be positive for infected *S damnosum* which have not yet been fully surveyed

(c) The table on page 548, giving a total of 1 400 is correct. It refers to both the Sué I and Sué II survey. This should be obvious, but the printer's spacing has slightly obscured it. Dr KIRK (1947) has referred to the same survey which we did together and the same total on page 358 of his paper.

I am etc.,

H. M. WOODMAN.

Juba,
Equatoria, Sudan.

7th January, 1950.

REFERENCE.

KIRK, R. (1947). *Ann. trop. Med. Parasit.* 41, 357.

GAPS IN THE KNOWLEDGE OF YAWS

SIR,

Dr C. J. HACKETT's paper "Gaps in the Knowledge of Yaws" (presented to the Seventh Pacific Science Congress, New Zealand, 1949) published in the TRANSACTIONS November 1949 was of great interest to us in Jamaica.

The gaps in our knowledge of yaws are not really as wide as the article would lead us to believe however. In fact, the clinical manifestations of the disease and its epidemiology have been surprisingly accurately described by such writers as WILLIAM HILLARY 1766, THOMAS DANCER, 1774 to 1810, JOHN WILLIAMSON 1817, WILLIAM WRIGHT 1828, TURNER *et al.* 1937, CHAMBERS 1938, who worked in Jamaica. The biggest gap in the knowledge of the disease was filled when CASTELLANI, in 1905 found the causative organism, and gave it the name *Spirochaeta* (later changed to *Treponema*) *pertenue*.

In 1934 while working with the Rockefeller Foundation, I pointed out that a warm climate and humidity were the major factors in the distribution and spread of yaws at any rate in Jamaica. In addition, I revised the names given to the various manifestations of the disease, particularly in the secondary stage. Later on I had these various lesions classified under the three stages of the disease in their most usual sequence. Lesions of the palms of the hand and the soles of the feet were not left uncorrelated with the course of the disease but were ascribed to the secondary stage and late (or rather delayed) secondary stage of the disease.

I should like to comment on each of the headings under which Dr HACKETT states that further investigations are required

(1) I agree that study of the antigenic characters of the respective organisms is necessary to give an answer regarding the identity or difference between the organisms causing yaws or syphilis

R L KAHN, in America, is carrying out further investigations in just this direction I am informed that he has already shown that the antigenic characters of serum from persons of the same race infected with yaws or with syphilis, show distinctive different serologic patterns

However, those workers, in my experience, who have seen a good deal of yaws and syphilis in the same race can readily differentiate between the clinical manifestations of the two diseases Most difficulty arises in lesions of the tertiary stage, and greater reliance has to be made on an accurate history This is not surprising as the tertiary lesions in both diseases represent an allergic reaction to the presence of a very small number, or perhaps the products from a small number, of one or other organism

(2) Dr HACKETT mentioned where YASUYAMA (1928) found that *Treponema pertenue* survived 2 hours in human serum, but did not mention that CHAMBERS (1938) showed survival up to 8 hours in serum at 84° F

(3) Dr HACKETT calls attention to the possible transmission by flies or inanimate objects, as did WRIGHT in 1828 In a study for source of infection in 62 consecutive primary lesions, I obtained a definite history of personal contact during the incubation period with infected cases in 95 16 per cent The two cases in which definite evidence of contact was not obtained, were of school children attending school, on the road to which school infectious subjects of yaws would be walking

(4) With regard to observations on congenital transmission, in no case among 129 children under 4 years of age seen with primary and early Stage II lesions, was there evidence of congenital transmission (See also CHAMBERS, 1937)

(5) Under heading Geographical Distribution, all the factors suggested for close study and correlation have been very closely studied in Jamaica Humidity was found to be the important factor (CHAMBERS, 1938) So much so is this the case, that I can drive through any area in Jamaica today and, by observing the climatic and geological factors of the area, state whether there will likely be any cases of yaws in that area or not, and, if present, whether the incidence will be high or low

(6) Detailed descriptions of the pathological changes in many lesions of yaws are inadequate, but this deficiency is being looked into by pathologists in Jamaica

stilbamidine, from L.D. bodies by 1 : 40 000 stilbamidine at 37° C. for 24 hours, and subsequent exposure to 24° C. stilbamidine was effective in a concentration of 1 : 50 000 in the case of flagellates and 1 : 300,000 in the case of L.D. bodies. These findings warrant the conclusion that L.D. bodies are more sensitive than flagellates to aromatic diamidines. Apparently the effect on oxygen uptake is not the only factor in the lethal action of these drugs on leishmania.

I am, etc.,

S. ADLER.

Department of Parasitology
Hebrew University
Jerusalem.

5th February 1950.

REFERENCES.

ADLER, S., TCHERNOMORETZ, I. & BER, M. (1948). *Ann. trop. Med. Parasit.* 42, 1.
FULTON, J. D. & JOYNER, L. P. (1949). *Trans. R. Soc. trop. Med. Hyg.* 43, 273.

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ANNOUNCEMENTS.

NEXT MEETING OF THE SOCIETY

The next meeting will be held at Manson House, 26, Portland Place, on Thursday, 18th May, 1950 Professor R M GORDON, of the Liverpool School of Tropical Medicine, will read a paper entitled "The Problem of Loiasis in British West Africa"

MANSON LECTURE

To perpetuate the memory of the late SIR PATRICK MANSON, the Council of the Society has decided to establish a MANSON LECTURE FUND, to which subscriptions are now invited It is hoped to raise a sum of at least £2,500, the accumulated interest from which will be devoted to financing a Manson Lecture

The Lecture will deal with some aspect of tropical medicine or hygiene and will be given periodically by a recognized authority The lecturer and the subject on which he will be invited to speak, will be decided by the Council of the Society

The Manson Lecture will be open to all members of the medical profession and will be advertised in the general medical press, in which it may be subsequently published.

MOVEMENTS OF FELLOWS

The following Fellows from abroad have notified the Secretaries that they are *temporarily* in the British Isles Letters addressed to any of these care of the Royal Society of Tropical Medicine and Hygiene, Manson House, 26, Portland Place, London, W 1, can usually be forwarded to the home address

To ensure the accuracy of this list, Fellows named below are particularly requested to advise the Secretaries when they return to their stations abroad

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BARNES, G T, Fiji
BEET, E A, N Rhodesia
BOSE, P N, India
BRAINE, G I H, Malaya
BUCK, S, N Rhodesia
BUNNY, A S, Kenya
CALWELL, H G, Tanganyika
CAMPBELL, G, Trinidad
CHANG, HOEY CHAN, Malaya
CRAN, D L, Sudan
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DAS, S K, India
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ELMES, B G T, Canada
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GELFAND, M S, Rhodesia
GOH, K A, Hongkong
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HUNTER, W, Nigeria
JACKSON, ROSEMARY, Tanganyika
KELSEY, H A, Nigeria
KENNEDY, Col T F, S Rhodesia
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LOW, NAN-WAN, Malaya
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SEEVARATNAM, V J, Malaya
SEKAR, S C, India.,
SIMPSON, T, Nigeria
SUR, M L, India
TAYLOR, WALTER, South Africa.
TO, SHIU-YUEN, Hongkong
UPTON, B H B, Fiji
VAN-DE LINDE, P A M, Hongkong
WALLACE, R B, Malaya
WHEATON, F L, Sudan
WILSON, D BAGSTER, Tanganyika.
YEO, K. C, Hongkong
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PROPOSER — 1 ________________ *Signature of Fellow*

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I hereby undertake, if elected, to observe and obey the Laws and Regulations of the Royal Society of Tropical Medicine and Hygiene, and to endeavour to promote its honour and interests.

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The submission of matter for publication will be understood to imply that it is offered to this journal alone.

If accepted for publication, the copyright of papers becomes the property of the Society, but they may be re-published by permission of the Council, provided due acknowledgment be made of their having appeared in the TRANSACTIONS.

Papers should, if possible, be typewritten; they should be concisely written with subject matter logically arranged and sub-divided, with references and abbreviations in the form described below, and with indications of the position, in the text, of illustrations, tables, maps, etc.

Titles should be as brief as consistent with clarity, and in many cases the value of a paper is enhanced by a short summary at the end.

Temperature charts, graphs and drawings should be, if possible, in Indian ink on Bristol board, with detail and essential lettering large enough to be clearly legible after reduction if necessary. (Write in pencil if lettering on a drawing is to be set up and printed.)

Illustrations—if the number sent in is considered excessive, the author may be informed and given the opportunity of contributing to the cost.

Coloured plates are made only at the authors' expense.

REFERENCES.

In the text, the date of publication, in brackets, should follow the name of the author quoted thus:—

T. MANSON (1878) made this epoch-making discovery.

At the end of the paper a list of References should be arranged in alphabetical order of authors' surnames, and details given in the following order:—(1) Surname of author; (2) Initials of author; (3) Year of publication, in brackets; (4) Title of article, avoiding arbitrary capitals. (The title of the article is sometimes omitted; but each list of references should in this respect be consistent throughout—giving all titles, or omitting all.) (5) Title of Journal; (6) Volume number; (7) Page number. *e.g.*:—MANSON, P. (1878). On the development of *Filaria sanguinis hominis* and on the mosquito considered as a nurse. *J. Linn. Soc. (Zool.)*, 14, 304.

In the case of a reference to a book: (1), (2) and (3) as above; (4) Title of book; (5) Edition and/or volume if more than one; (6) Page number; (7) Town of publication; (8) Publishers' name. *e.g.*:—MANSON, P. (1898). *Tropical Diseases*, 1st Ed., 447. London: Cassell & Co. Ltd.

Reference to an Annual Report: SWAZILAND (1937). *Annual Medical & Sanitary Report 1936*, p. 16.

Note: The year of publication is not usually the year covered by the Report.

ABBREVIATIONS.

The abbreviations used are those shown in the **World List of Scientific Periodicals, 1934**, which conforms to the rules of the *International Code of Abbreviations for Titles of Periodicals*, Paris, 1930. In general, nouns have capitals, adjectives small, initial letters; articles, conjunctions and prepositions are omitted; the place of imprint is added only when uncertainty might arise, *e.g.*:

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Ann. trop. Med. Parasit.	*C. R. Acad. Sci., Johannesburg.*	*Ned. Tijdschr. Geneesk.*
Arch. Schiffs- u. Tropenhyg.	*Dtsch. med. Wschr.*	*Trans. R. Soc. trop. Med. Hyg.*
Bull. Soc. Path. exot.	*Indian med. Gaz.*	*Z. Hyg. InfektKr.*

The following contractions are in use whether the number to be expressed is 1 or more (*e.g.* 1 c.c., 15μ, 45 kg.):—

centigramme, cg.	kilometre, km.	millimetre, mm.
centimetre, cm.	micron, μ.	ounce, oz.
cubic centimetre, c.c.	microgrammes, μmg.	pound, lb.
cubic millimetre, c.mm.	milligramme, mg.	
kilogramme, kg.	millilitre, ml.	

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TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE.

VOLUME 48

MAY, 1950 No 6

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NOTICE

The attention of Fellows is drawn to *Laws of the Society*, Rule 8 (para 3) "Fellowship of the Society has no status as a diploma or academic credential No Fellow shall make use of the designation F R S T M & H in this sense after his name"

[The previous number of these Transactions, Vol 43, No 5 was published on 24th March, 1950]

TRANSACTIONS
OF THE
ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

VOL 43 No 6 MAY, 1950

ORDINARY MEETING

of the Society held at
Manson House, 26, Portland Place, London, W ,
on
Thursday, 16th February, 1950, at 7 30 p m

THE PRESIDENT,
Professor H E SHORTT, C I E, M D, D SC, D T M & H, COL I M S (RET),
in the chair

THE THIRD ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE CHADWICK LECTURE

CHLORAMPHENICOL (CHLOROMYCETIN) AND TROPICAL MEDICINE

BY

JOSEPH E SMADEL,
From the Department of Virus and Rickettsial Diseases, Army Med ' Department Research and Graduate School, Washington, D C

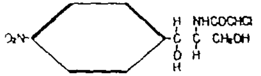
The story of chloromycetin, or to use its generic nai.. hloramphenicol, is one which illustrates the progress in medicine which sometimes results from the close co-operation of a number of groups of scientists from different fields Botanist PAUL R BURKHOLDER, of Yale, recovered the organism which yielded the antibiotic subsequently named chloromycetin (EHRLICH *et al*, 1947), BARTZ (1948), EHRLICH, SMITH and other bacteriologists (1947, 1948), of the Parke, Davis Research Laboratories, prepared crystalline chloromycetin, and showed that it inhibited a wide range of microbial agents Investigators at the Army Medical Department Research and Graduate School found the new substance possessed marked activity against the rickettsial group of agents

(SMADEL and JACKSON 1947) and that its oral administration to man resulted in appreciable levels of drug in the body fluids without producing toxic manifestations (LEY *et al.* 1948). All was ready in 1947 for progress along two distinct paths. One of these the clinical application of the antibiotic to the treatment of infectious diseases of man, will be discussed in considerable detail in this talk. The other path, the biochemical one, followed by CROOKS and his associates (CONTROULIS *et al.*, 1949 REBSTOCK *et al.* 1949) led to the discovery of the structural formula of the antibiotic and to its synthesis, first as a laboratory feat but shortly afterward as a successful commercial method of production.

The structural formula of chloramphenicol is rather simple. According to the research chemists of the Parke Davis Laboratories, this is the first recognized naturally occurring compound produced by living tissue which contains a nitro group or which is a derivative of dichloracetic acid.

The results of laboratory studies provided strong hope that chloramphenicol might prove to be the specific therapeutic agent which had long been sought

CHLORAMPHENICOL (CHLOROMYCETIN)

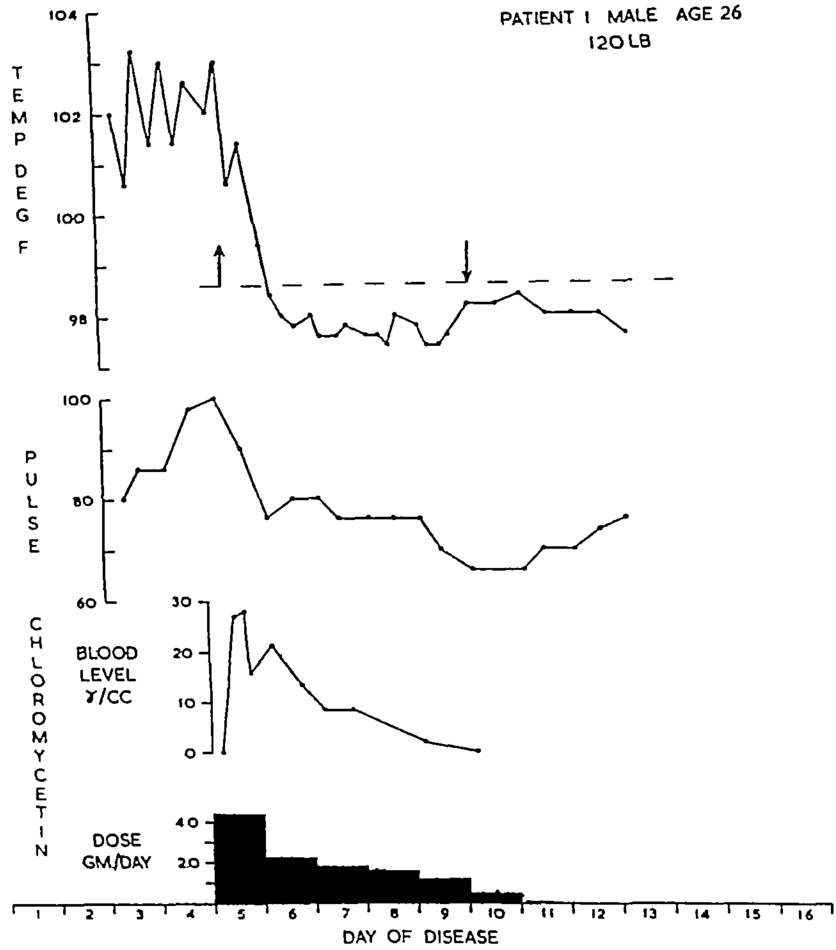


THREO- -DICHLORO-ACETAMIDO-PARA-NITROPHENYL- [illegible]

FIG. 1—Structural formula of chloramphenicol. (Reproduced from REBSTOCK *et al.*, *J. Amer. Chem. Soc.* 1949)

for the rickettsial diseases. Because treatment of this group of infections was still relatively unsatisfactory the first clinical trials of the new drug were made on patients with epidemic or endemic typhus. These initial investigations during the winter of 1947–48 in Mexico (SMADEL, LEON, *et al.*, 1948) and Bolivia (PAYNE, *et al.*, 1948) provided much needed experience in the use of the drug in patients and sustained the hope generated in the laboratory The next step required a carefully controlled study in which the apparent efficacy of chloramphenicol in patients with typhus fever could be critically evaluated.

For an investigation of this type to be successful many things are required. There must be available not only adequate clinical material but also satisfactory hospital and laboratory facilities. The Army Research group thought that Kuala Lumpur might provide these essentials for the study of scrub typhus. Dr LEWTHWAITE, then Director of the Institute for Medical Research, replied with enthusiasm to our inquiry and arrangements were completed for a series of collaborative studies to be carried out in Malaya. I wish that all such joint investigations could be as pleasant and productive as these have been.



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
RASH			+++	++	+	O	O									
ESCHAR			+	+	+	+	+	+	±	O						
BP						104/[illegible]0	100/70	98/[illegible]	10[illegible]/[illegible]0	96/70	104/70	110/70	12/74			
WBC (THOUSANDS)								6 0								7 2
RBC (MILLIONS)								4 0								4 2
WF OX-K					80		320		1280		5120				5120	
RICKETTSEMIA				+	+	+	O									

FIG 2—Response of scrub typhus Patient 1 to chloramphenicol (Reproduced from SMADEL, WOODWARD, LEY & LEWTHWAITE J clin Invest 1949)

TREATMENT OF PATIENTS WITH SCRUB TYPHUS.

On the day of arrival of the American group in Kuala Lumpur chloramphenicol therapy was instituted on the first patient, a soldier of one of the British Malay regiments, who had the typical clinical picture of scrub typhus. A graphic summary of his clinical record is given in Fig. 2.

The dramatic response of this individual is typified by the temperature curve. Fever disappeared by crisis and the temperature was normal 20 hours

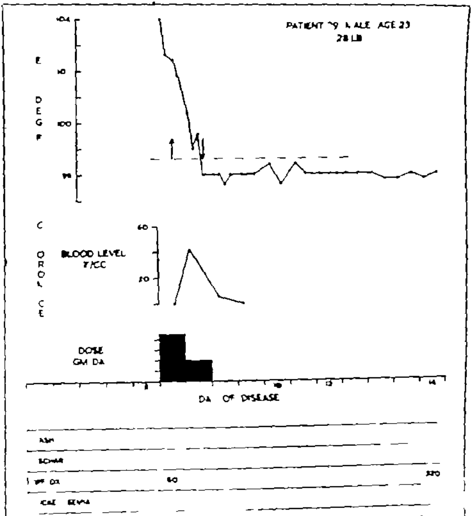


Fig. 3.—Response of scrub typhus Patient 29 to chloramphenicol. (Reproduced from Smadel, Woodward, Ley & Lewthwaite, *J. clin. Invest.*, 1949.)

after chloramphenicol therapy was instituted Other manifestations of illness diminished at the same time as the fever, except the primary eschar which required 5 days for healing It is worth directing attention to the line at the bottom of the chart Circulating rickettsiae were demonstrated by inoculation of animals with blood taken from the patient immediately before treatment, 12 hours later while the patient was still febrile, and also 30 hours after therapy was started at which time the patient was afebrile and asymptomatic Other clinical records, to be shown later, will demonstrate this same phenomenon of rickettsemia unaccompanied by clinical manifestation of illness The antibiotic was administered by the oral route to this patient and to all others whom we have treated It was our practice to give an initial dose of gramme 3 0 of drug, and follow this by gramme 0 25 at intervals of 2 or 3 hours In this instance treatment was continued for a total of 5 days

Recovery was so rapid that it seemed unnecessary to prolong chemotherapy for a number of days Accordingly, the duration of treatment was progressively shortened The record of Patient 29, summarized graphically in Fig 3, illustrates the results obtained when gramme 5 0 to 6 0 were given over a period of 24 hours While our group obtained satisfactory responses in a number of scrub typhus patients who were treated with a single oral dose of chloramphenicol gramme 4 0, we were not bold enough to recommend such a procedure for general use Subsequently, Captain GILES and Major SYMINGTON (1949), of the Military Hospital in Malaya, employed single 3 0-gramme oral doses of the antibiotic in 13 soldiers with scrub typhus and found this regimen to be adequate

The results obtained in the first 30 patients with scrub typhus who were treated with chloramphenicol are summarized in Fig 4 along with correspond-

TABLE I —SCRUB TYPHUS PATIENTS, KUALA LUMPUR 1948

	Treated	Untreated
Number of patients	30 23 males 7 females	19 16 males 3 females
Day after onset recipe begun	3 to 11 Average 6 2	
Last febrile day of illness	4 to 12 " 7 4	12 to 31 Average 17 1
Duration of fever after recipe begun (hour)	6 to 96 31 8	
Day after onset discharged from hospital	14 to 28 17 8	17 to 51 Average 29 9
Complications	0	1 parotitis 1 pneumonia
Deaths	0	1 17th day
Month of onset	March-Sept	Feb -June

FIG 4—Summary of results in first series of 30 treated cases of scrub typhus (Reproduced from SMADEL, WOODWARD LEY & LEWTHWAITE *J clin Invest* 1949)

areas, and these were usually heavily infested with vector mites. Finally the *Trombicula akamushi* and *T. deliensis* collected from these wild rats were known to harbour *R. tsutsugamushi*. The infection rate among the mites was undoubtedly high since in a number of instances pools of less than 25 *T. ombicula* yielded strains of rickettsiae when inoculated into mice.

The results of the first chemoprophylactic field test are summarized in Fig. 5. Seventeen of the 24 volunteers in the control group developed scrub typhus between the 12th and 21st days after initial exposure in the infected areas. These persons were promptly admitted to hospital and given specific therapy. Each individual was subsequently proved to have suffered from scrub typhus by the demonstration of the presence of rickettsaemia during the febrile period or by the development of a positive Weil Felix reaction during convalescence or by both procedures.

The 22 members of the test group remained well throughout the period of prophylaxis and for the following week. At this point, on the 28th day after initial exposure, we congratulated ourselves on a successful experiment and dismissed the volunteers. To our chagrin 3 days later scrub typhus began to appear among the members of the test group. And within the week, 12 of the 22 persons who had received chemoprophylaxis were admitted with scrub typhus. The disease in these persons differed in only one respect from that previously observed in members of the control groups. None of those who had received prophylaxis developed primary eschars whereas almost 30 per cent. of the controls showed this lesion.

Four additional chemoprophylactic field trials, each patterned after the first, have now been completed. While each provided some information, only the first and third were very fruitful (SMADEL, TRAUB *et al.* 1949 1950). The others failed to answer certain of the specific questions under investigation because of the low infection rates among volunteers of the control groups. Nevertheless, these failures provided definite information on the epidemiology of the disease and on the ecology of the vector mites.

The results of Trial 3 clearly indicated that chemoprophylaxis of scrub typhus could be attained. In this instance chloramphenicol was given in weekly oral doses of gramme 4·0 for a period of 4 or 6 weeks after the volunteers had been exposed for 6 days in hyperendemic areas of scrub typhus. The data presented in Fig. 6 show that 13 of the 18 members of the control group developed scrub typhus between the 10th and 14th days after initial exposure. Volunteers in Group II received a total of 4 weekly doses of drug beginning at the end of the exposure period. Members of Group III were given a total of 6 weekly doses of drug, the first of the series being administered 4 days after the last exposure in the field. Among the 31 volunteers who received prophylactic chloramphenicol only one developed classical scrub typhus and required hospital detention during the period of administration. Equally important, none of the remaining 30 volunteers developed clinical disease after the course of prophylaxis was completed.

Even though 30 of the volunteers in the prophylactic groups of Trial 3 were not in hospital, many of them displayed evidence of smouldering infection During the time when prophylactic doses were given, a number developed eschars of scrub typhus and almost half had short intermittent periods of mild fever which lasted for a day or so These febrile episodes usually occurred about the time the next chemoprophylactic dose was due, and the temperature promptly returned to normal shortly after the drug was given During these mild febrile phases, while the patients were still afoot, the majority of the volunteers in the prophylactic group, who were tested, were found to have rickettsemia Furthermore, OX-K antibody titrations, which were done on

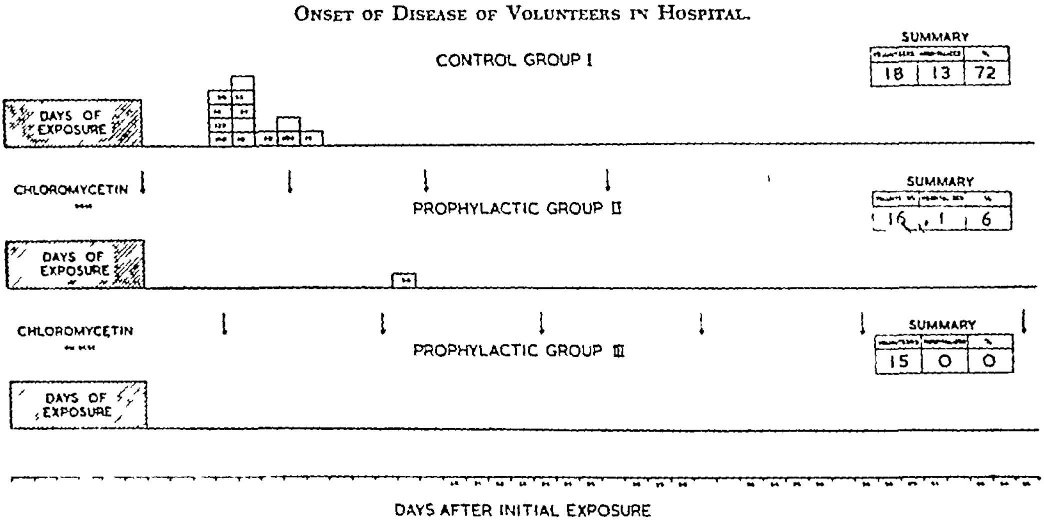


FIG 6—Chemoprophylactic effect of chloromycetin against scrub typhus in volunteers Trial No 3 Kuala Lumpur December, 1948

specimens of sera taken before the experiment and after the end of the test, revealed that significant amounts of OX-K antibodies developed in about two-thirds of the test volunteers Thus, the infection rate in the volunteers who received chemoprophylaxis was about the same as it was in the control group

Such experiments as those mentioned indicate that the chemoprophylaxis of rickettsial diseases is feasible However, the general usefulness of this preventive measure is limited by practical considerations At the present time it would find its main application in protecting military personnel or certain plantation workers who are heavily exposed to scrub typhus for definite periods of time Chemoprophylaxis of rickettsial diseases probably has no place as a public health measure at the moment in the United States or in Europe

You will recall that vaccination against scrub typhus is unsatisfactory. The successful chemoprophylaxis and treatment of scrub typhus opens up a new avenue of approach for regularly inducing active immunity against this disease in human beings. It is now perhaps feasible to immunize people by inoculating them with a living attenuated strain of *R. tsutsugamushi* and to prevent the development of clinical disease by the proper chemoprophylactic administration of chloramphenicol. We intend to investigate this subject in the near future.

The chemoprophylactic trials indirectly provided new information on the host parasite-drug relationship (SMADEL, BAILEY and DIERCKS, 1950). This concerned the occurrence of relapses of scrub typhus in about half of the 56 volunteers who had contracted scrub typhus and who had apparently responded satisfactorily to specific therapy. It should be emphasized that this phenomenon of recrudescence had never been observed in patients who acquired scrub typhus during normal occupational duties; this was true irrespective of whether they suffered the full-blown disease under symptomatic treatment or an abbreviated illness under chloramphenicol therapy. The relapses almost invariably occurred during the second week after onset of disease. Furthermore the average interval between the first dose of drug given for the initial attack and the recurrence of fever was approximately 7 days.

Such recrudescences of scrub typhus in volunteers were promptly controlled when another course of chloramphenicol was given. Fig. 7 summarizes graphically the record of one of the patients whose findings illustrate this point. Numerous factors which might contribute to the relapses were considered and some were investigated either in the wards or in the laboratory. The immediate problem was solved rather simply, however, by giving a supplementary dose of chloramphenicol as a prophylactic measure at about the time when a relapse was to be expected.

The record of Patient 143 illustrates an instance in which the supplementary dose of drug was given at an opportune time. The patient was asymptomatic and afebrile on the ninth day after onset of disease when chloramphenicol gramme 4·0 were administered. Nevertheless at this time he had demonstrable *R. tsutsugamushi* in his blood. It may be stated with assurance that this volunteer was in the initial stage of a relapse when the drug was given and that clinical disease was suppressed as a result of treatment.

We were not always so lucky in our selection of the time to give the supplementary drug. Patient 539, whose record is presented in Fig. 9, illustrates such an instance. Here it was planned to give the suppressive therapy on the morning of the ninth day after onset of disease. Through an error this dose was not administered until the evening and at that time the patient had a moderate elevation of temperature and some constitutional reaction. One might say that the result was therapeutic rather than prophylactic. At any rate the relapse was rapidly controlled.

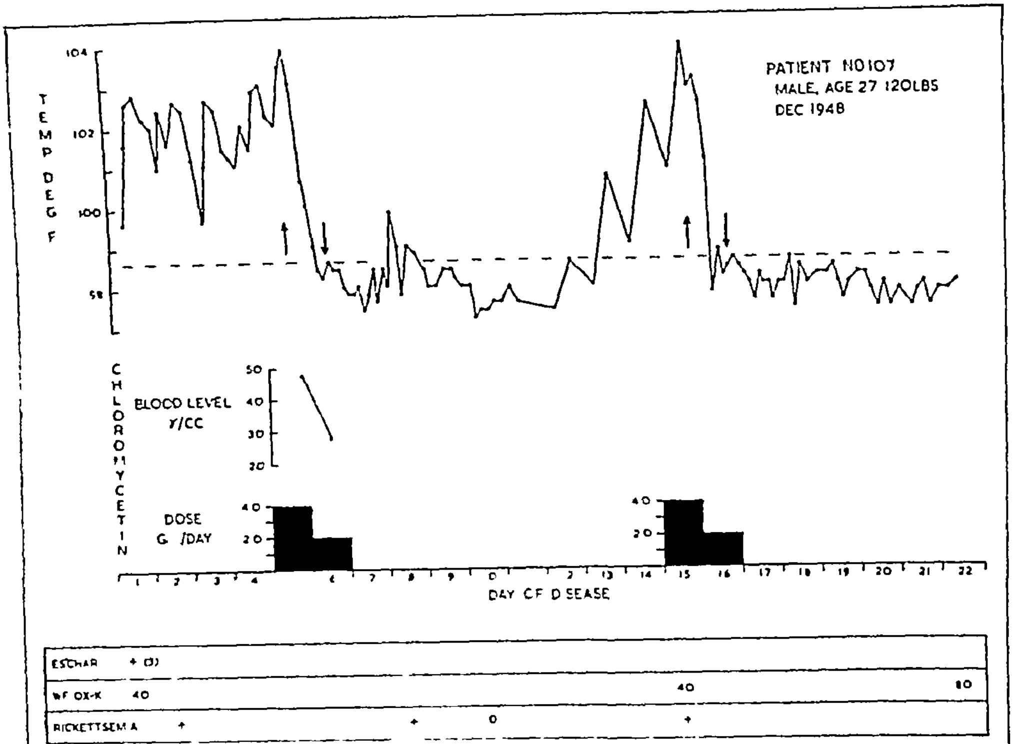


FIG 7—Volunteer 107 whose relapse of scrub typhus responded to a second course of chloramphenicol (Reproduced from SMADEL, BAILEY & DIERCKS, *Amer J Hyg*, 1950)

MODE OF ACTION OF CHLORAMPHENICOL IN SCRUB TYPHUS

Many of the observations discussed early in this report have provided insight into the mode of action of chloramphenicol It might be desirable at this point to review these data and to supplement them with observations from the laboratory

All information pointed to the fact that chloramphenicol has a rickettsiostatic action and not a rickettsiocidal one Thus, active rickettsiae continued to circulate in the blood of patients for an appreciable time after treatment was begun Moreover, rickettsemia was demonstrable in ambulatory volunteers during the course of chemoprophylaxis Furthermore, certain patients, *i e*, volunteers in control groups who contracted scrub typhus, suffered relapses after an interval of apparent cure And finally, the volunteers who received chemoprophylaxis for only 2 weeks after exposure came down with full-blown scrub typhus a week after the drug was discontinued It was clear that chloramphenicol *per se* did not eliminate the rickettsiae from the patient Experimental studies corroborated this (SMADEL, JACKSON and CRUISE, 1949) For example,

mice infected with *R. tsutsugamushi* and treated daily with full therapeutic doses of chloramphenicol for a period of 3 months, still harboured rickettsiae in their tissues. *In vitro* tests provided additional evidence. *R. tsutsugamushi* was unaffected by soaking in solutions containing ten times the maximal concentration of chloramphenicol attained in the blood of patients.

The evidence which pointed toward the idea that chloramphenicol suppressed the multiplication of rickettsiae is so conclusive that I shall not reiterate the findings or augment them with laboratory experience. However there is an intriguing question which concerns the duration of the suppressive effect. Why does a single dose of drug suppress clinical signs of disease for approximately a week? The figure of 7 days is taken from the observations on relapses, on the onset of clinical disease in volunteers who received the short course of

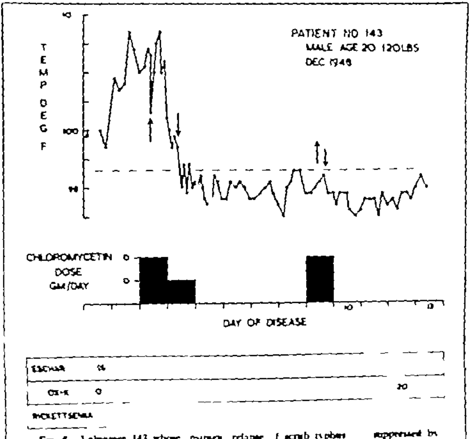


FIG. 6.—Volunteer 143 whose incipient relapse of scrub typhus was suppressed by supplementary dose of drug. (Reproduced from SMADEL, BAILEY & DIERCKS, Amer. J. Hyg., 1950.)

prophylaxis, and on the mild cyclic fevers in the ambulatory volunteers who received drug weekly in the long course of prophylaxis The 7-day period of suppression is an average, actually the extremes were 5 to 11 days Chloramphenicol is rapidly excreted from the body and is no longer detectable in the blood 24 hours after a single oral dose of gramme 3 0 to 4 0 Therefore, the persistence of appreciable amounts of the native antibiotic cannot account for the prolonged therapeutic effect There are a number of theoretical possibilities which might be considered in explaining this prolonged period of suppression, but in the absence of any factual data it appears fruitless to discuss them

The observed 7-day period of suppression of clinical signs of scrub typhus which results when chloramphenicol is given can be used as a cornerstone to explain the occurrence of relapses in treated volunteers with scrub typhus, and their absence in treated patients who had acquired the disease under natural conditions Uncomplicated and untreated scrub typhus characteristically runs a 14-day course Rickettsemia can be demonstrated regularly during the first 8 or 9 days of the illness and in some instances as late as the 11th day Therefore, it would appear that the immune mechanism of the patient begins to become

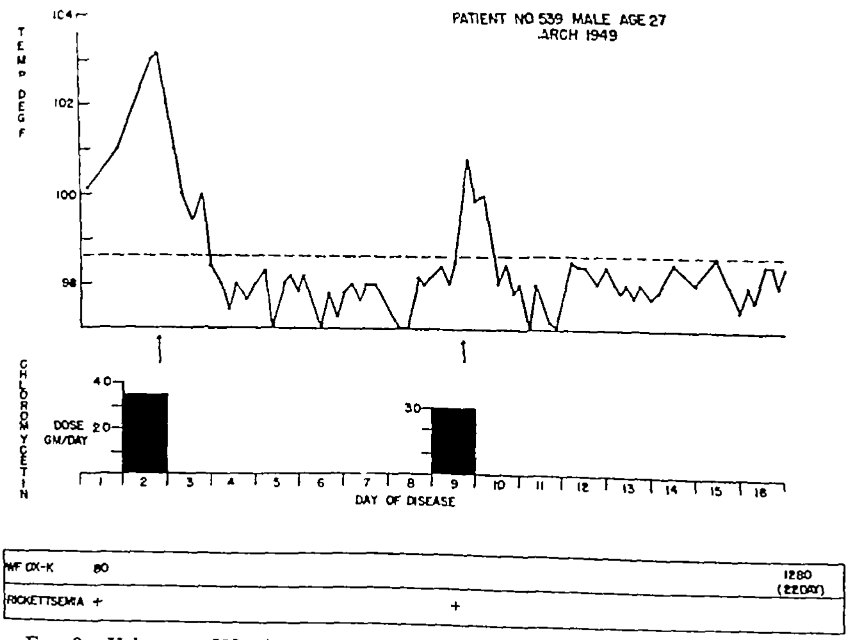


FIG. 9—Volunteer 539 whose clinical relapse which had just begun when supplementary dose was given was aborted by treatment (Reproduced from SMADEL BAILEY & DIERCKS, *Amer J Hyg*, 1950)

effective during the mid-portion of the second week of illness and that the factors of resistance gain the ascendency over the rickettsial agent by the 14th day Patients with the natural disease often are not available for treatment during the first week of illness. Indeed, the mean value for the day on which therapy was begun in 43 such patients was 7 1 In contrast, the mean day on which treatment of the 62 volunteers who contracted scrub typhus was started was 3·4 If the suppressive effect of specific therapy lasts for 1 week, it would be expected that most of the natural cases and few of the volunteers would have acquired adequate immunity before the drug effect was lost. There are a number of factors which undoubtedly contribute to the phenomenon of relapses in volunteers, but the simple explanation just given probably includes two of the most important of these.

One might logically ask at this point why prophylaxis must be continued for 4 weeks after exposure when 2 doses of drug properly spaced, are adequate to control the initial disease and suppress the impending relapse in exposed volunteers who develop clinical disease It is my opinion that the answer is probably to be found in the qualifying phrase "properly spaced." Perhaps an appreciable amount of rickettsial antigen is required in order to obtain an immune response adequate to control the infection. If such is the case, then the necessary stimulus may not be obtained when one induces an almost complete suppression of growth of *R. tsutsugamushi* Perhaps the organism must at times slip out from under the oppressive effect of the drug and undergo some multiplication in order to provide the required amount of antigen. Such an hypothesis may be untenable tomorrow however it at least recognizes that a delicate balance exists between the host, the organism, the drug, and the immune state. The recognition of such a balance appears necessary

This relatively long discussion on the mode of action of chloramphenicol in scrub typhus leads up to a simple conclusion. Chloramphenicol does not "cure the patient in the sense that it destroys the rickettsiae instead it suppresses the multiplication of the organism while the patient develops his immune and defence mechanisms in the usual way and these control the infection permanently

Before leaving the subject of mechanism, it might be mentioned that we considered the possibility that chloramphenicol might have a direct detoxifying effect on the toxin of *R. tsutsugamushi* but we were unable to produce experimental evidence to support such an idea.

The title of this talk is concerned with chloramphenicol, but it should be pointed out that aureomycin produces the same therapeutic results in this disease as does chloramphenicol (SMADEL, BAILEY and DIERCKS, 1950). Both drugs result in equally rapid defervescence. Relapses have not been observed in the rather small group of persons with naturally occurring scrub typhus who received aureomycin but have been seen in volunteers who contracted the disease. These relapses were amenable to treatment when aureomycin

was again administered Furthermore, a supplementary dose of this antibiotic suppressed the relapse if given at the appropriate time

TREATMENT OF TYPHOID FEVER

When the American group arrived in Malaya it had no intention of working on anything except scrub typhus The Parke, Davis investigators had given the Army team almost every available gramme of chloromycetin and we were determined to husband every milligramme of this hoard in order to maintain a reserve adequate for the treatment of all volunteers in the chemoprophylactic trials, should the infection rate be high enough to require this expenditure

Despite the care exercised in selection of patients who were to receive the drug, some did not have scrub typhus and valuable stocks were "wasted" I put quotation marks around the word wasted and make no apology for the fact that 15 of the first 49 cases selected were not proved to have scrub typhus Laboratory procedures were invaluable for the final confirmation of diagnosis of tsutsugamushi disease but were of no immediate assistance during the first week of the disease Needless to say, it was desirable to treat the patients early in the course of their illness rather than late in the second week Among the 15 patients with missed diagnosis there were three who had murine typhus Each of these recovered promptly on chloramphenicol therapy, this confirmed our early experience in Mexico Of greater importance to the present discussion was the fact that two members of the group were subsequently proved to have typhoid fever It was the favourable response of these individuals which led to the obtaining of additional supplies of drug and to more detailed studies on the use of chloramphenicol in the treatment of typhoid fever (WOODWARD *et al*, 1949, 1950)

The graphic record of Patient 5, reproduced in Fig 10, illustrates the usual findings in typhoid fever treated with chloramphenicol The beneficial effects of therapy do not become evident as rapidly in this disease as in scrub typhus In fact, little improvement is noted during the first 2 days of treatment However, by the third or fourth day the fever had abated by lysis, the rose spots, if originally present, have disappeared, and the headache, cough, and toxemia have ameliorated From this point onward convalescence usually proceeds at a rapid rate The bacteriological findings in this patient were similar to those of the majority who were treated early in the course of disease *Salmonella typhosa* was cultivated from the blood prior to treatment of this patient but not after the drug was administered The specimens of urine and faeces examined did not yield typhoid organisms in this instance

Analysis of the records of 45 of our patients with typhoid fever who were treated with chloramphenicol during the initial course of their disease has shown that 44 survived The average duration of fever in these 44 patients was approximately 4 days after drug therapy was instituted Most of the group were treated before the 21st day of illness and the majority of these were given

the drug during the second week. However essentially similar results were obtained irrespective of whether therapy was given relatively early or relatively late in the disease.

All of the adults with typhoid fever received an initial oral dose of gramme 3·0 followed by gramme 3·0 daily until the temperature became normal, and then by gramme 1·0 or 2·0 daily for a variable period of time. The first patients were given the total daily amount of drug in divided doses at 2- or 3-hour intervals while the fever persisted, and at 4- to 6-hour intervals thereafter.

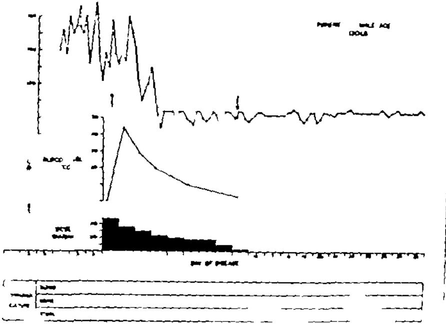


Fig. 10.—Patient 5—usual response of typhoid fever patient to treatment with chloramphenicol. (Reproduced from Woodward et al., J. [illegible] 1950.)

It is now evident that a number of the patients seen early in the study received inadequate treatment. The graphic record of Patient 6, shown in Fig. 11, illustrates the usual course of disease in the seven patients who suffered a relapse of typhoid fever. Patient 6 responded satisfactorily to the course of chloramphenicol which was begun on the 12th day and stopped on the 19th. Headache, fever and bacteremia reappeared on the 31st day, which was 16 days after the patient had become afebrile and 12 days after the last dose of drug. Another course of chloramphenicol was prescribed; the patient responded rapidly and made an uneventful recovery. Among the seven patients in this group, the relapses occurred 8 to 16 days, average 11, after therapy was stopped.

Or, stated another way, these episodes began between the 26th and 44th days average 32, after onset of disease It is worth mentioning that the strains of *S typhosa* recovered from the blood stream of patients during the relapse were as sensitive to chloramphenicol as were the organisms isolated before treatment was begun

When it was suspected that the high relapse rate was due to inadequate treatment, and when sufficient chloramphenicol became available, the course

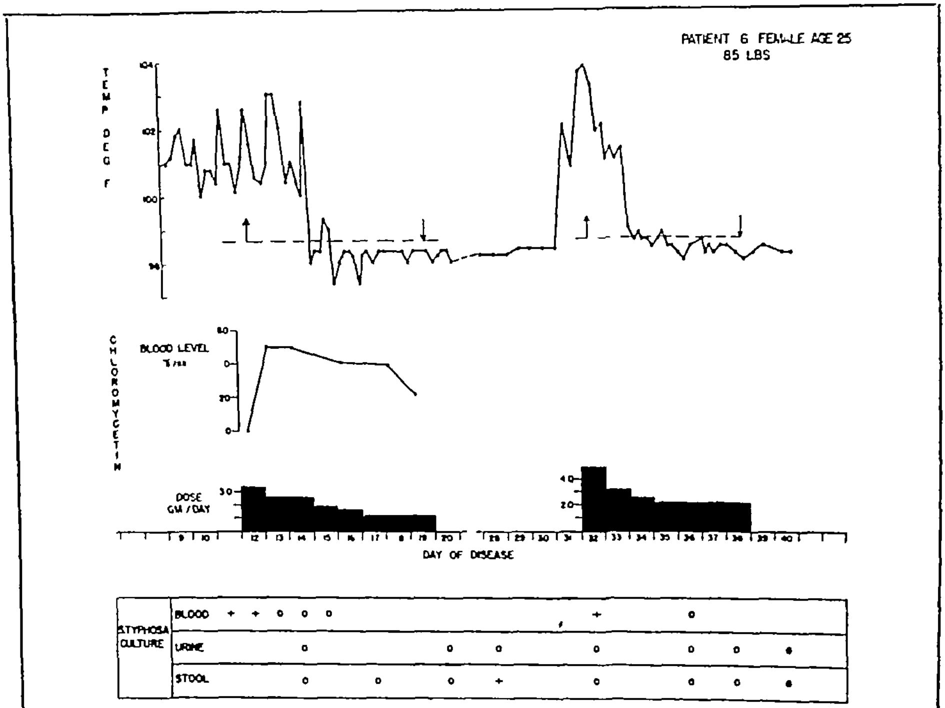


Fig 11—Patient 6—following apparent recovery the patient suffered a relapse of typhoid fever which responded when chloramphenicol was again administered (Reproduced from Woodward *et al*, *J clin Invest*, 1950)

of therapy was gradually prolonged The data on 44 treated patients, summarized in Fig 12, indicate that a high relapse rate may be expected if the drug is given for 8 days or less and that few relapses may be encountered if it is given for 14 days (Smadel, Woodward and Bailey, 1949) It is still too early to set a standard schedule for treatment of typhoid fever However, the last one employed by our group in Malaya (Smadel, Bailey and Lewthwaite, 1950) was as follows an initial oral dose of gramme 3 0 or 4 0 was given, followed by gramme 1 5 at 12-hour intervals during the febrile period, then gramme 1 5 in a single daily dose for 7 days, followed by gramme 1 0 daily until the

14th day. More recently Dr. T. E. Woodward, clinician of our group and senior author of our first reports on typhoid fever, has employed interrupted treatment somewhat analogous to that used to prevent relapses in the volunteers with scrub typhus. The first 5 days of this regimen are similar to that just mentioned, the drug is then omitted for 5 days after which the original course is repeated. He observed no relapses in the eight Americans with typhoid fever who were maintained on this regimen.

Chloramphenicol therapy has not eliminated the two common complications of typhoid fever which are intestinal haemorrhage and intestinal perforation. If one recalls the typical typhoidal ulcer of the ileum with the entire mucosa sloughed off and the necrotizing process extending into and at times through

Patients.		Treatment with chloramphenicol.					Relapses.	
		Duration of treatment.		Administration.				
				Day of disease.		Total grammes given (average).	Number of patients affected.	Per cent. of patients affected.
Group.	Number.	General period.	Total days (average).	Started (average).	Stopped (average).			
A	13	8 days or less	6.9	13.6	20.4	20.0	7	53.8
B	10	9 to 14 days	11.2	13.7	24.9	23.7	0	0
C	13	Longer than 14 days	18.0	20.6	38.8	3 .8	0	0

Fig. 12.—Relation of relapses of typhoid fever to duration of treatment. (Reproduced from Smadel, Bailey & Lewthwaite, Amer. J. Med. 1950.)

the muscularis, then one may expect that accidents will continue until sufficient time has elapsed for regeneration and repair of tissue. How long a time is required for such healing in treated patients is unknown, but 1 week after institution of therapy would appear to be sufficient. Four of our 45 patients had an intestinal haemorrhage between the second and seventh days after chloramphenicol was administered; this was between the ninth and 22nd days of disease. In two instances haemorrhage occurred before the temperature had returned to normal and in the two others within the first 3 days of the afebrile period.

Two of the typhoid patients had perforation of the intestine but recovered. Each of these accidents occurred on the 21st day of disease, which was the third or fourth day of treatment. One patient in the group of 45 succumbed. He died on the 18th day of illness after 4 days of treatment. He had repeated

intestinal haemorrhages, intestinal perforation, and pneumonia In discussing these cases, it is worth pointing out that chloramphenicol (and for that matter aureomycin also) is extremely valuable in controlling the generalized peritonitis which usually follows perforation None of these three patients received the benefit of surgical intervention Signs of generalized peritonitis disappeared in 4 or 5 days in the two who survived, while in the third, who died, autopsy revealed only a small area of local peritonitis in the immediate vicinity of the perforation

Patients with typhoid fever who are treated with chloramphenicol still require the usual isolation precautions but they are less of a menace to the public health than are untreated patients Urine and faeces from many of our cases never contained demonstrable *S typhosa* Some who yielded positive cultures before therapy did not again supply such samples after treatment was begun, a few excreted the organisms on several occasions during the course of drug or shortly afterward, several of the patients again excreted *S typhosa* during their clinical relapses All eventually provided a series of negative specimens before discharge from the hospital

It was a great disappointment to find that chloramphenicol failed to eradicate the chronic typhoid-carrier state This should have been expected, however, in spite of the efficacy of the drug in the acute disease Chloramphenicol in concentrations of a few gamma per c c in culture media will inhibit the growth of *S typhosa*, but even a thousand gamma will not kill the organisms The typhoid carrier is, from one point of view, an immune individual who has settled his personal problem with the invading bacterium and the two now live happily together In other words, the balance between the host, the parasite, and the immune mechanism is already established, and the added factor of a transient suppressive, such as chloramphenicol, is unlikely to produce much in the way of a permanent effect on the bacterium

It would appear that many of the points mentioned in the discussion of the mode of action of chloramphenicol in scrub typhus are also applicable in typhoid fever In both instances the drug suppresses the pathogen and gives the host time to develop his mechanisms of resistance Ultimate recovery results from the response of the host, not from the direct effect of the antibiotic on the invader

During the year and a half since the appearance of the first report on the specific therapeutic action of chloramphenicol in typhoid fever, the drug has been widely used by others in this disease In general, their experiences have been similar to those I have recounted

I have already spent more than my allotted time and have discussed in detail only two diseases, yet the title included the broad field of tropical medicine Mention must be made of the efficacy of chloramphenicol in the rickettsial disease, Rocky Mountain spotted fever, and in the venereal diseases of varied aetiology, *viz*, gonorrhoea, lymphogranuloma venereum, lymphogranuloma inguinale, and syphilis Similarly, the benefits derived from the drug in patients

with brucellosis and tularemia are worthy of note. Laboratory studies suggest that a number of other infectious diseases of man may be helped by this antibiotic but clinical investigations remain to be done before conclusions are warranted.

REFERENCES.

BARTZ, Q R. (1948). Isolation and Characterization of Chloromycetin. *J biol Chem* 172 445.

CONTROULIS, J REBSTOCK, M. C. & CROOKS, H. M., JR. (1949). Chloramphenicol (Chloromycetin). V Synthesis. *J Amer chem. Soc* 71 2463

EHRLICH, J BARTZ, Q R., SMITH, R. M., JOSLYN D A. & BURKHOLDER, P R. (1947) Chloromycetin, New Antibiotic from Soil Actinomycete. *Science* 106, 417

GILES, H McC. & SYMINGTON T (1950) Chloromycetin in Scrub Typhus. *Lancet* 1 16.

LEY H L., JR., SMADEL, J E. & CROCKER, T T (1948). Administration of Chloromycetin to Normal Human Subjects. *Proc. Soc exp Biol Med.*, 68 9

PAYNE, E. H., KNAUDT J A. & PALACIOS, S (1948) Treatment of Epidemic Typhus with Chloromycetin. *J trop Med. Hyg* 51 68.

REBSTOCK, M. C., CROOKS, H. M., JR., CONTROULIS, J & BARTZ, Q R. (1949). Chloramphenicol (Chloromycetin). IV Chemical Studies. *J Amer chem. Soc* 71 2458

SMADEL, J E. & JACKSON E. B. (1947). Chloromycetin, an Antibiotic with Chemotherapeutic Activity in Experimental Rickettsial and Viral Infections. *Science* 106 418.

——— LEÓN, A. P LEY, H. L., JR. & VARELA, G (1948). Chloromycetin in the Treatment of Patients with Typhus Fever *Proc. Soc. exp. Biol. Med.*, 68 12.

——— WOODWARD, T E., LEY H. L., JR., PHILIP C. B., TRAUB, R., LEWTHWAITE, R & SAVOOR, S. R. (1948) Chloromycetin in the Treatment of Scrub Typhus *Science* 108 160

——— JACKSON, E. B. & CRUTZE, A. B (1949). Chloromycetin in Experimental Rickettsial Infections. *J Immunol.*, 62, 49

———, TRAUB, R. LEY, H. L. JR., PHILIP C B. WOODWARD T E. & LEWTHWAITE, R (1949) Chloramphenicol (Chloromycetin) in the Chemoprophylaxis of Scrub Typhus (Tsutsugamushi Disease). II Results with Volunteers Exposed in Hyperendemic Areas of Scrub Typhus. *Amer J Hyg* 50 75

——— WOODWARD T E. & BAILEY C. A. (1949). Relation of Relapses in Typhoid to Duration of Chloramphenicol Therapy *J Amer med. Ass* 141 129.

——— ——— LEY H. L., JR. & LEWTHWAITE, R. (1949). Chloramphenicol (Chloromycetin) in the Treatment of Tsutsugamushi Disease (Scrub Typhus) *J clin Invest.* 28 1196.

——— BAILEY C. A. & DIERCKS, F H (1950) Chloramphenicol (Chloromycetin) in the Chemoprophylaxis of Scrub Typhus (Tsutsugamushi Disease). IV Relapses of Scrub Typhus in Treated Volunteers and their Prevention. *Amer J Hyg* (in press).

——— ——— & LEWTHWAITE, R. (1950) Synthetic and Fermentation Type Chloramphenicol (Chloromycetin) in Typhoid Fever Prevention of Relapses by Adequate Treatment. *Ann int Med.* (in press).

——— TRAUB, R FRICK, L. P DIERCKS, F H. & BAILEY C. A. (1950). Chloramphenicol (Chloromycetin) in the Chemoprophylaxis of Scrub Typhus (Tsutsugamushi Disease). III. Suppression of Overt Disease by Prophylactic Regimens of Four Weeks Duration *Amer J Hyg* (in press)

SMITH, R. M JOSLYN D A. GRUHZIT O M., MCLEAN I W JR., PENNER, M. A. & EHRLICH, J (1948). Chloromycetin Biological Studies. *J Bact* 55 425

WOODWARD T E., SMADEL, J E., LEY H. L., JR GREEN R. & MANKIKAR, D S (1948). Preliminary Report on the Beneficial Effect of Chloromycetin in the Treatment of Typhoid Fever *Ann. int. Med* 29 131

——— ——— ——— (1950). Chloramphenicol and Other Antibiotics in the Treatment of Typhoid Fever and Typhoid Carriers. *J clin Invest* (in press)

Discussion

Dr R Lewthwaite I should like to thank Dr SMADEL for the very kindly remarks that he made about the welcome we gave him I think that you will agree, after the masterly exposition of his subject that he has just given us, that our decision to receive a unit from his laboratory in Washington was fully justified It was not a difficult decision to make Firstly, the protocols of his experiments with chloromycetin and scrub-typhus in mice and fertile hen-eggs, appeared full of promise, especially those recording late therapy Secondly, scrub-typhus had menaced the people of Malaya in the past, especially the planting community, and still more so the British and American armies in the Japanese war Thirdly, many of us here have had the opportunity to visit the United States, and have come back with a lively appreciation of the high calibre of the American laboratory workers and their unstinted generosity in demonstrating to us, and debating with us, not only past but also current work In Kuala Lumpur, at the Institute for Medical Research, we work rather on the fringe of the world of medical research, and I and my professional colleagues, 18 in number, and comprising clinicians, experimental pathologists and bacteriologists, entomologists, biochemists and nutrition workers, realized, perhaps selfishly, the immense profit that would accrue to each one of us academically by the presence among us of five hand-picked American medical research workers It took just 24 hours for a cable to go back to Colonel RUFUS L HOLT, Dr SMADEL's Commandant, extending a warm welcome to the project

Concerning the clinical and laboratory data so lucidly put before us by Dr SMADEL tonight, I can only, as an accomplice before and during the event, commend them to you, and note that they formed the basis of a project that was carried through with the speed and dynamic energy which I suspect comprise Dr SMADEL's normal tempo of work.

I should like to dilate on the many thrills and buoyant good humour that punctuated this investigation, but must be brief First of all there was the arrival of a huge United States Army Transport plane Five American scientists having disembarked, one keeping a watchful eye on the world's supply of chloromycetin (viz, 1 lb), and frigidaire, hot-air sterilizers and incubators, etc, having followed, there remained, neatly tucked in at the back of the plane, two recalcitrant United States Army jeeps In Manila they had been emplaned with electric hoists in a matter of minutes But looted Singapore fell far short of such amenities, and eventually only girders and ropes and a bevy of perspiring labourers succeeded in dislodging and landing them I should say that no aeroplane was ever in such jeopardy of physical mutilation by scientists as was this one, nor was scientific endeavour ever "grounded" for 24 hours for so unique a reason

Then, in Kuala Lumpur, on the day of arrival at the laboratory, began a vital 48 hours, following the administration of the drug to two patients in

the adjacent hospital both severe cases of scrub-typhus. What would happen? If the drug failed to have any favourable effect on these two patients, then as one of the American Unit remarked, that Unit would have seriously to consider returning there and then to the United States. In 48 hours the temperature was normal and remained normal, and toxicity had vanished. Immediately came the problem of whether these patients were indeed cases of scrub-typhus. Few of us had any doubt but recovery of the causal *Rickettsia* in the mice inoculated with blood was essential for absolute proof and a tense 10 days followed. No inoculated mice ever received greater attention than did these. They duly succumbed.

Another thrill was the arrival in July 1948, of a test-tube containing 11 25 gramme of what was referred to as "a new type of chloromycetin" which one or two of us knew to have been obtained by synthesis. The amount was barely enough for two patients. These we obtained they were two Gurkha soldiers infected naturally during military operations. Both were treated with the synthetic drug both responded precisely as patients treated with the fermentation type of drug had done. We thus had witnessed an event which was then unique in antibiotic therapy

A third episode which I would like to mention was the organization by Dr SMADEL of the field trials in which human volunteers were exposed. These comprised Americans, British, Malays, Chinese and Indians but the Malays formed the bulk. They left at 7 o clock in the morning by lorry for the infected area 13 miles away N.A.A.F.I canteens went out with food at 11 a.m. nd 3 p.m. and at 6 p.m. the motley throng returned. The strain of 10 consecutive days of tropical sun was no light one but the young Malays whiled away the time with their guitars, playing cards, etc and their absolute trust that if they contracted the disease the drug would cure them wa testified by the considerable waiting lists for the next four field experiments.

There was of course more in the planning of these field trials than the mere letting loose of volunteers into a field. The trials were made with all the precision of a scientific experiment. Thus the two entomologists f the United States Unit had sampled for mites certain selected circumscribed areas w thin the field by placing, over night or longer numbers of white rats contained within suitable wir netting domes. These rats were then brought into the laboratory any mites removed and identified, and from the species and number it was pos ble to ensure that the large multi-coloured umbrellas (borrowed from the N.A.A.F.I.) were placed, with four olunteers beneath them, in heavily infested areas. I might mention, in passing the enthusiast who took 70 mites from his shoes and put them down his vest his zeal was rewarded! A mite trap devised by Mr K. L. COCKINGS, of the British Scrub-Typhus Unit, was also used t spot f vourable areas. Another refinement came from the observations by the United States entomologists that the mites were most active as the dew was lifting or settling and hours were shortened accordingly without loss of effect

The time is getting late, and I feel that I should not say too much because, as you will have gathered from Dr SMADEL's remarks, I was in amongst it, and I see many distinguished scientists here today to whom I should hand over the rostrum as soon as possible. But before doing so, I should like to mention one very recent development in the treatment of a disease which has a virus of the psittacosis-lymphogranuloma venereum group, namely, trachoma. In the last number of the *British Journal of Ophthalmology* there is an article by Mr BOASE, the Government Ophthalmologist in Kampala, Uganda. Sir STEWART DUKE-ELDER, during a recent visit to East Africa, had given him a supply of aureomycin with the object of assessing it in trachoma. When I visited his clinic in November last, he drew my attention to six cases that he had treated by external instillation of this antibiotic drug. He was immediately struck by the very rapid improvement that resulted. In so much as both psittacosis and lymphogranuloma venereum respond to aureomycin, it is perhaps not surprising that trachoma should be favourably affected. Mr BOASE later used chloromycetin orally in one case (having no more drug), and he obtained promising indications. It looks, therefore, as though yet another recalcitrant virus disease may yield to antibiotics.

Sir Alexander Fleming: I have got nothing serious to contribute to this discussion, but I would like to congratulate Dr SMADEL on his masterly exposition. This Society has had many important communications, but very few have been more important than this one. Here is another disease conquered. We do not see much typhus here, it is not a serious disease in England, but to people working in laboratories, and those who got mixed up with making typhus vaccines during the war, typhus fever was a serious business, and there were disasters. Now there is no fear at all: if you get typhus you get cured with a little chloromycetin or you can have a prophylactic dose once a week. You can play with typhus without being frightened of it. I have not had any experience of chloromycetin in tropical diseases. We have used the drug in urinary and other infections: most of them yield to chloromycetin, even those by proteus and pyocyaneus, which are resistant to most things. Then there was a paper the other day about chloromycetin being used for rapidly sterilising the upper respiratory tract. We confirmed this in a patient who was being treated for ulcerative colitis. The streptococci and other organisms disappeared from the throat but in a day or two they were replaced by a coliform bacillus and the patient complained of a sore throat. Apparently the disappearance of the normal flora in the throat and the substitution of other organisms disturbed the patient.

Antibiotics are gradually getting into their stride. Ten years ago we had only one, 5 years ago we had two, and now there are four good ones and others in the offing. Where it will end we do not know, but chloromycetin has made one big break in the antibiotic field. It is the only one commercially synthesized, and it is to be hoped that this synthesis will bring down the price.

Sir Howard Florey It is very kind of you to ask me, a visitor to say something Perhaps I am one of the few people here besides Drs. LEWTHWAITE and SMADEL, who had the opportunity of seeing this work. Dr LEWTHWAITE very kindly invited me, on my way back from Australia, to see the investigations on scrub-typhus at Kuala Lumpur The skill with which this work was carried out has been mentioned. I can back up Dr LEWTHWAITE when he says that the organization of Dr SMADEL's party was extremely good I can also say that that of Dr LEWTHWAITE's department was the same. The atmosphere which prevailed during this investigation is illustrated by the fact that Dr SMADEL was known to most people as Joe!

Before the work in the field could be done there was much preliminary investigation by a large commercial firm in the States. They had the assistance of many investigators of its biological properties. One communication on chloromycetin impressed me particularly there was half a page of acknowledgements to workers in various parts of the U.S.A. to whom the substance had been sent for investigation. There was thus a very fine organization behind both the original laboratory investigations and the field trials, which sets a first-class example of what should be done in this field. If chloromycetin had turned up in this country no one would have known about its action against rickettsiae as there is apparently no one working on them here at the moment. That is worth thinking over

One point that I had not realized before and which to me is very interesting is that chloromycetin does not kill but only stops growth even of sensitive bacteria. Some people have had the view that chemotherapeutic agents should kill, especially bacteria, if they are to be effective but that does not seem to be the case. I think, from the theoretical point of view that it is extraordinarily interesting that the body does a good part of the protective work.

But I do not want to waste your time I would merely like to pay a tribute to Dr SMADEL and his co-workers and leave it at that.

Dr A Felix It is a privilege and a pleasure to join the previous speakers in congratulating Dr SMADEL, Dr LEWTHWAITE and their colleagues on their splendid achievement. Those of us who are old enough to have witnessed the amazing progress in our knowledge of the fevers of the typhus group, between the first and second World Wars, are in a position to assess the magnitude of this achievement. One need only recapitulate the many unsuccessful attempts at chemotherapy of louse-borne typhus that were made during the last war German workers reported early during the war that trials with nearly 100 different drugs including 24 sulphonamide compounds gave only negative results (WOHLRAB, 1942). French workers in Tunis held the view that sulphapyridine was not only useless but even harmful (DURAND and BALOZET 1941) The British team at Hampstead tested some 238 drugs without definite success, and the U.S.A. Typhus Commission, of which Dr SMADEL was a member have not been more fortunate in their trials.

said tonight is of general application I must say that I was very much struck by a remark that Dr SMADEL made towards the end He defined chloromycetin as a transient suppressive, and I have a feeling that the word suppressive is appropriate to many, perhaps most, other chemotherapeutic agents All that we do by administering these agents is to interfere with or suppress the activities of the infecting micro-organisms, perhaps only for a short time, and we are dependent on the defence mechanisms of the host for the eradication of the infection When we apply chemotherapy we have always to look after and support the patient in such a manner that he himself can finally kill off the micro-organisms and get on with the process of repair I consider that Dr SMADEL, besides showing us some very beautiful experiments on the clinical application of chloromycetin, has made a most valuable contribution to our knowledge of the processes involved in the chemotherapeutic treatment of infections

Dr G M Findlay As the chemotherapeutic activities of aureomycin have been mentioned, it may be of interest to point out that aureomycin has a remarkable curative action in phagaedenic tropical ulcer and in secondary yaws, two of the most widespread and most common of all diseases in the tropics In tropical ulcer aureomycin by mouth causes a complete disappearance of the pus containing spirochaetes and fusiforms in 48 hours with subsequent healing of the ulcers In secondary yaws aureomycin by mouth produces drying up of the lesions in about 7 days Aureomycin by mouth is slightly slower than penicillin by injection but, like penicillin, it does not influence the serological reactions A paper by Dr OKU AMPOFO and myself, giving a preliminary account of these investigations, is now in the press

Professor R Cruickshank We are interested in the fundamental action of chloromycetin on bacteria, and we were early impressed with the effect which the drug seemed to have on *Ps pyocyanea* in chronic urinary infections, producing abnormal colinial variants which could not readily be recognized

Following these findings, Dr A VOUREKA has been studying the effect of chloromycetin on coliform bacteria When the drug is used alone, it has no very marked effect, but combined with the specific antiserum, bizarre forms are formed which behave like true mutants Since these abnormal forms would presumably be quickly destroyed by the host's phagocytic cells, we must not judge the value of a drug by its behaviour in the test tube In the case of chloromycetin, its therapeutic activity probably depends to a considerable extent on the presence of specific antibodies, and Dr SMADEL's experience of relapses in typhus lends support to this view

I wonder if he is right in his suggested explanation for the failure of chloromycetin to cure chronic typhoid carriers The inaccessibility of the typhoid bacillus to the drug may be an important factor, since there is evidence

It would be very tempting for me to continue to discuss this important immunological problem, but I must resist because our President might stop me and I would like to say a few words about chloromycetin in typhoid fever

I am afraid the results so far obtained in typhoid fever are less striking than those in typhus fever It is true the drug has considerable effect on some of the constitutional symptoms of typhoid fever and this, of course very much impresses the clinician. On the other hand, the drug in the doses used up to the present, often has no effect, or very little effect, on the excretion of typhoid bacilli in the faeces and also has no effect on the relapse rate which is very high. Naturally this side of the picture very much impresses the bacteriologist.

Dr SMADEL referred to the disappointing finding that the drug did not eradicate the chronic typhoid-carrier state once this was established. Unfortunately the treatment apparently also fails to prevent the development of the carrier state In a small outbreak in this country last year (CROWTHORNE April, 1949) affecting about 40 persons the only persistent excreter that was identified was one of the female patients who had received two courses of chloromycetin treatment. She has now been excreting for 9 months, and unfortunately she is very likely to remain a chronic carrier It is true the dosage employed during that trial which has not yet been published in detail, was the one originally suggested by WOODWARD and his colleagues (1948), and this is now considered to be inadequate. In a more recent outbreak (in Salford, October 1949), the interrupted courses of treatment, now recommended by Dr WOODWARD have been employed, and according to preliminary reports from the two bacteriologists concerned, the effect of this treatment on the excretion of typhoid bacilli was not better than that of the original regimen

I may perhaps be permitted to plead with the distinguished clinicians present here tonight for the closest co-operation between clinician and pathologist in the planning of future therapeutic trials. This co-operation has not been very conspicuous in some of the papers published in this country yet it is essential for the solution of the problem f the chronic carrier which is of paramount importance from the public health point of view

In conclusion, I should like again to congratulate D SMADEL and Dr LEWTHWAITE

REFERENCES.

DURAND, P & BALOZET L. (1941) *Arch Inst. Pasteur Tunis*, 30 1
FELIX, A (1942) *Brit med J* 2 597
——— & BHATNAGAR, S S (1935) *Brit J exp Path.*, 16 422.
WOHLRAB R (1942) *Klin Wschr* 21 455
WOODWARD T E SMADEL J E LEY H L GREEN R & MANKIKAR, D S (1948) *Ann intern Med.* 29 131

Dr F R Selbie I feel it rather embarrassing to be called upon to speak here because I have no experience either of typhus or of chloromycetin. But I am int rested in chemotherapy nd I think that much that Dr SMADEL has

said tonight is of general application I must say that I was very much struck by a remark that Dr SMADEL made towards the end He defined chloromycetin as a transient suppressive, and I have a feeling that the word suppressive is appropriate to many, perhaps most, other chemotherapeutic agents All that we do by administering these agents is to interfere with or suppress the activities of the infecting micro-organisms, perhaps only for a short time, and we are dependent on the defence mechanisms of the host for the eradication of the infection When we apply chemotherapy we have always to look after and support the patient in such a manner that he himself can finally kill off the micro-organisms and get on with the process of repair I consider that Dr SMADEL, besides showing us some very beautiful experiments on the clinical application of chloromycetin, has made a most valuable contribution to our knowledge of the processes involved in the chemotherapeutic treatment of infections

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I wonder if he is right in his suggested explanation for the failure of chloromycetin to cure chronic typhoid carriers The inaccessibility of the typhoid bacillus to the drug may be an important factor, since there is evidence

that most drugs cannot easily enter a non-functioning gall bladder such as is often present in the chronic typhoid carrier But if the drug gets access to the organism in the presence of antibody the pathogen should quickly be destroyed.

One last point since these new antibiotics attack viruses, and probably bacteria inside cells, one wonders what effect they may have on tissue cells generally There is a need for long-term studies on the pharmacology and possible injurious effect of these drugs.

Dr Smadel (in reply) I would like to thank each of the discussors for his remarks they have contributed materially to the subject reviewed this evening

I wish to comment specifically on only a few of the questions which were raised. In our experience the Weil-Felix test using OXK antigen, is superior in scrub-typhus to the complement-fixation test employing specific rickettsial antigen. Among the 100-odd cases of scrub-typhus, including volunteers who contracted the disease, we were able to establish the diagnosis by isolation of *Rickettsia tsutsugamushi* in approximately 90 per cent. About 80 per cent. of the entire group developed significant increases in OXK antibody during convalescence Slightly less than half of the members of the group whose sera have been employed in complement fixation tests with rickettsial antigen have yielded positive reactions. There are marked variations in the antigenic composition of different strains of *R. tsutsugamushi* This antigenic heterogeneity probably accounts for the low incidence of positive complement fixation reactions. The problem is still under investigation in our laboratory

The problem of relapses in patients with typhoid fever who were treated with chloramphenicol undoubtedly requires additional study However it is apparent from the data presented in the text that its importance has been greatly reduced since the recognition of the value of continuing therapy for 10 days or so after the patient becomes afebrile.

The accumulated evidence to date warrants the following statements about the development of the carrier state in patients with typhoid fever who were treated with chloramphenicol (1) The incidence of carriers is no higher in treated patients than in those who receive no specific therapy in the latter instance 1 to 3 per cent. of surviving patients become carriers and (2) an occasional typhoid patient who recovers after chloramphenicol therapy becomes a carrier of *Salmonella typhosa*. Thus, at the present time it would appear that chloramphenicol neither increases the incidence of carriers nor does it prevent the development of typhoid carriers.

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COMMUNICATIONS

EXPERIMENTS WITH "ANTRYCIDE" IN THE SUDAN AND EAST AFRICA

BY

D G DAVEY

Imperial Chemical Industries, Limited, Biological Laboratories, Hexagon House, Manchester, 9

The experiments described below started in January, 1948, and continued until April, 1949 They are concerned almost entirely with the curative and protective action of "Antrycide"* (which in its early history was called M 7555) against infections in cattle with *Trypanosoma congolense* and *T vivax* An addendum to the report mentions briefly some results achieved against other species in other hosts A full account of the events leading to the development of antrycide, and of the experiments that were done with it in small laboratory animals, is published elsewhere (CURD and DAVEY, 1949, 1950), and only those features of the early work which are relative to an understanding of the field experiments will be discussed here

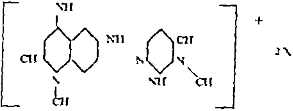
The field work was made possible through the co-operation and help of the Colonial Office, the High Commission for East Africa and the East African Veterinary Research Organization, the Government of the Sudan and the Sudan Veterinary Service, the Government of Uganda and the Uganda Veterinary Department, and the Kenya Veterinary Department I planned the experiments, but many individuals helped with advice about local conditions and in the actual carrying out of the work I am indebted particularly to Mr J T R EVANS, of the Sudan Veterinary Service, Mr R N T-W-FIENNES, of the East African Veterinary Research Organization, Dr E A LEWIS, of the Kenya Veterinary Department, and my assistants, Mr H MOORES and

* Trade Mark of Imperial Chemical (Pharmaceuticals), Ltd

Mr A. S. TAYLOR. I also acknowledge with gratitude the help which was received from Dr E. G. WHITE, of the East African Veterinary Research Organization, Mr W. LANGRIDGE of the Kenya Veterinary Service, Mr H. CROWLEY, Dr S. G. WILSON, Mr D. HOPKINS and Mr S. G. LAWS of the Uganda Veterinary Service, and my colleague Mr J. S. STEWARD. Each experiment is the joint work of myself with one or more of these individuals.

THE PROPERTIES OF ANTRYCIDE

The substance has the constitution



where X is Cl (antrycide chloride) or CH_3SO_4 (antrycide methylsulphate).

These two salts, the chloride (M.P. 316° decomp.) and the methylsulphate (M.P. 265-266°), both of which are white crystalline solids, are the only two that have been used in the experiments. They appear to have equal trypanocidal activity once they are brought into contact with trypanosomes, but they possess widely different solubilities in water which give them different pharmacological properties. The methylsulphate is soluble to the extent of about 33 per cent. in water, whereas the chloride is soluble only to the extent of about 0.12 per cent. Absorption of the two salts after subcutaneous or intramuscular injection appears to be directly proportional to their solubilities and so suspensions of the chloride are absorbed slowly and poorly, and solutions of the methylsulphate rapidly and fairly completely. This was clearly to be inferred from the early biological experiments, and it has since been amply confirmed by Dr A. SPINKS, a colleague in these laboratories, who has evolved a method of estimating antrycide (SPINKS, 1949), and who has followed the absorption and excretion of the two salts in laboratory animals and in calves. SPINKS (personal communication) has shown, for example, that a subcutaneous dose of 5 mg. per kg. of antrycide chloride in calves gives a maximum concentration in the plasma after 2 hours of only about 40 μg. per litre, which falls to 10 μg. per litre within 24 hours, whereas an equivalent subcutaneous dose of the methylsulphate gives a maximum concentration in the plasma of 2,700 μg. per litre, which again falls to 10 μg. per litre within 24 hours.

The differences in biological properties between the methylsulphate and the chloride that are a consequence of their differing solubilities may be summarized as follows:

(i) A solution of the methylsulphate is more toxic than an equivalent suspension of the chloride when it is given subcutaneously or intramuscularly. For example mic

have been given more than 1 gramme per kg and rabbits more than 200 mg per kg of the chloride in suspension without any marked general toxic effect, whereas the M L D of the methylsulphate in mice is about 15 mg per kg

It is a point of importance that mixture of the methylsulphate with a solution containing chlorine ions will lead to precipitation of antrycide chloride The toxicity of the two salts when given intravenously will therefore be about the same if due allowance is made, of course, for the different molecular weights

(ii) In the curative treatment of small laboratory animals such as mice the methylsulphate and the chloride give the same results, whatever the route of administration, because the curative dose is so small that even the chloride will be in solution With bigger animals, particularly with the bigger domestic animals, so much more drug is administered that if the chloride is used it will be in the form of a suspension Differences between the results achieved using the methylsulphate and the chloride in the treatment of the larger domestic animals are therefore to be expected

(iii) The early biological work showed that the subcutaneous administration of antrycide chloride to laboratory animals protected them for some considerable time against attempts to infect them with trypanosomes It is believed that these prophylactic properties are dependent on the establishment of a reservoir of the drug in the subcutaneous spaces from which absorption is slow and spread over a long period

The fluid in the subcutaneous spaces will contain chlorine ions derived from sodium and potassium chloride, and it is to be expected that some conversion of antrycide methylsulphate into antrycide chloride will take place at this site after subcutaneous injection Now if the prophylactic properties of antrycide are dependent on the poor solubility and the poor absorption of the chloride salt, and not to its persistence in the blood system in the way, for example, that suramin (antrypol) persists, then the prophylactic properties of the methylsulphate will depend on how much conversion to chloride takes place in the subcutaneous spaces

The Trypanocidal Properties of Antrycide

Tables I and II summarize the results obtained with antrycide in curative and prophylactic experiments with various species of trypanosomes in mice Some results obtained with suramin and dimidium bromide in curative experiments are also given in Table I for comparison

What is substantially the information given in Tables I and II was communicated to members of the Colonial Office Tsetse Fly and Trypanosomiasis

TABLE I Approximate minimum curative dose in mg per kg of antrycide, suramin (antrypol) and dimidium bromide against various species of trypanosomes in mice

Drug	*T rhodesiense* (Tinde strain)	*T equiperdum*	*T equinum*	*T evansi* (Sudan strain)	*T congolense* (Busimbi strain)
Antrycide (S C)	12.5–25	0.5	1	2.5–5	1–2
Antrypol (I P)	5–10	2–4	2	25–50*	>100
Dimidium bromide (I P)	>25	>25	>25		2–4

* This strain of *T evansi* has probably been made resistant to antrypol through mistreatment of camels in the Sudan , other strains are much more susceptible and may be cured by 2–5 mg per kg

TABLE II. Prophylactic effect of antrycide in mice against attempted infection with *T. congolense* (Busimbi strain).

Dose mg. per kg. antrycide chloride	Results—weeks after treatment.				
		4	6	8	10
25	10/10—	[illegible]—	[illegible]—	7/[illegible]— 1/[illegible]+	1/[illegible]— [illegible]+
12.5	[illegible]—	[illegible]—	1/7— 6/7+	[illegible]+	
5	3/10—† 7/10+	[illegible]+			

i.e. 10 of 10 mice resisted infection.
† *i.e.* three of 10 mice resisted infection, seven of 10 mice became infected.

Committee towards the end of 1947 and with the co-operation of the Committee and of the Sudan Government arrangements were made for experiments to be done in East Africa and the Sudan. The reasons underlying the plan of the experiments will be appreciated from a study of Tables I and II and the account of the properties of antrycide given above.

THE PLAN OF THE FIELD EXPERIMENTS.

(i) Before going to Africa no information was available concerning the action of antrycide in any of the larger domestic animals beyond the results of a few toxicity experiments with the chloride in cattle. Preliminary information had first to be obtained therefore, that the drug was actually active in the larger domestic animals, and this was done in the Sudan. With the knowledge that the drug was active, the experiments were extended to Uganda and Kenya to embrace other strains and other environmental conditions.

(ii) Although it was expected, for the reasons already given that the methylsulphate would yield superior curative results, and the chloride superior prophylactic results these expectations had to be confirmed experimentally and so both salts figure in all series of tests. In some of the earlier tests more animals received chloride than methylsulphate because supplies of the soluble salt were restricted during this period.

(iii) In the beginning when a strict comparison was being made between the methylsulphate and the chloride allowance was made for the difference in molecular weights by multiplying the actual weight of the methylsulphate salt by the factor 1.32. Later when it became clear that the differences between the two salts were so considerable that a fine comparison was unnecessary the

practice was dropped It is indicated in the tables whether the figures given for the methylsulphate represent the real weight of substance or whether they should be multiplied by 1 32

(iv) The literature concerning animal trypanosomiasis in Africa is misleading in the sense that *T vivax* tends to be ignored, at least in chemotherapeutic experiments, and consequently the impression is given that this species is of little consequence For example, trypanocidal substances sent to Africa for trial have rarely been tested against *T vivax*, and in the case of M & W 1553 (dimidium bromide), although the drug is quite widely used in areas where *T vivax* is known to exist, and although 5 years or so have passed since it was sent to the field, even yet there appears to be little, if any, well documented information concerning its action on this species

The impression that *T vivax* is of small consequence is reflected in the early plans for these experiments Originally, in the Sudan and in Uganda, the experiments were planned to embrace mainly *T congolense*, and it was only after I had been in Africa for some time and realized that *T vivax* was almost, if not equally, as widely distributed as *T congolense* that an attempt was made to set up adequate experiments with *T vivax* Some information was then obtained concerning the action of antrycide against this species but, because of the delayed start, it is not so complete as that for *T congolense*

One thing is certain—*T vivax* is common in the Southern Sudan, Uganda and Kenya, and so the question of its importance must be decided by its virulence for domestic stock

(v) We were committed to test antrycide in a fairly complete way against *T evansi* in camels, but for the rest our main effort was concerned with cattle trypanosomiasis, and it was only as opportunity offered that infections in other hosts were studied Our experiments showed that antrycide could be used to cure camels of *T evansi* and also to confer a marked protection on them More information was needed, however, before recommendations for field use could be made, and so Mr J T R Evans, of the Sudan Veterinary Service, has extended the experiments He will publish detailed information on all the experiments together, but to make this report more complete I have added an addendum in which the main results with *T evansi* are summarized, and in which the results with other species are recorded

THE CATTLE USED IN THE EXPERIMENTS

These were of mixed origin In the experiments in the Sudan short horn Zebu were used , in Uganda, Ankole with some Zebu, and in Kenya a wide variety of mixed European breeds and also Zebu All these breeds were readily susceptible to infection with trypanosomes Whether or not there were shades of difference in susceptibility between them could not be judged because there were too many other variable factors in the experiments

CURATIVE EXPERIMENTS.

Criteria of Cure

Everyone knows what is meant by cure, but it is almost impossible to lay down criteria for it which are practicable and free from criticism. The most one can do is to justify criteria adopted under a particular set of circumstances. In these experiments an animal has been considered cured if following treatment blood smears were consistently negative for 112 days after treatment. Blood smears were usually read daily except Sundays. The great majority of them were thick smears made from blood taken from the ear vein and stained by the method described by LAWS (1931). At least 100 fields but more often two or three times this number would be examined. In some of the experiments some of the apparently cured animals were also subinoculated to confirm their freedom from infection.

On the whole, it is probably safe to say that the great majority of animals satisfying these criteria, particularly those infected with *T. congolense* were actually cured. This conclusion is reached from a consideration of the following facts

(i) The course of infection in animals having *T. congolense* and left untreated is, for the most part, very constant. Of 21 animals examined for 3 weeks or longer (up to 8 weeks) 19 exhibited trypanosomes in the blood every day. Their number varied from about one per 100 microscopic fields (thick stained smear 1/12" objective, x6 eyepiece) to about 10 or more per field. The fluctuation is illustrated by the following series of daily readings taken from a control animal in the Sudan

5/1 1/50 1/5, 1/2, 1/2, 1 10 1/2, 2/1 1/5 2/1 1/2.

The exceptions were animals infected with the Kenya T.90 strain. One sometimes gave smears apparently free of trypanosomes for several days on end and the other after a lengthened prepatent period (9 days instead of 6 or 7) only occasionally showed trypanosomes in the blood, these occasions being separated by phases of apparent negativity lasting 1 2 or 3 weeks.

The course of infection with *T. vivax* is much less constant. Generally speaking, phases when trypanosomes are apparent in the blood alternate with phases when they are not. A succession of daily readings from two untreated animals in the Sudan illustrate this.

443 10 1	1 5 1/2, 10 1 5 1 20 1 >20 1 >20 1 20 1
44 1 5	1 5 5 1 5 1
443 (contd.)	1 10 1 5 5 1 1 20 5 1
442 (contd.)	1 5 2 1 10/1 1 20

It appears that an "average" vivax behaves in this way but certainly one and possibly two extremes were encountered in these experiments. In th

All slide readings throughout the report are given as trypanosomes per microscopic fields, thus 1 50 means that roughly one trypanosome per 50 fields was found

work in Kenya the so-called Emali strain, which is maintained by Dr E A LEWIS by passage through *Glossina pallidipes*, was used, and this seems to kill most untreated animals within 2 to 3 weeks (seven controls, used at different times, all died within this period), and during the time the cattle are alive trypanosomes are easily observed in the blood The other possible extreme is a strain met with in the experiments in Uganda The strain was probably derived from game in the Musoli Forest near Entebbe (the donor cattle were grazing near the periphery of the forest), and it appears that in some infections with this strain the apparent negative periods may be far longer than those illustrated from the Sudan records Further details of this strain are given in the account of the curative experiments with *T vivax*

(ii) The course of the first relapse infection compared with the untreated infection may be modified by some immunity acquired by the host, but the modification as seen in an examination of blood smears is not very considerable To illustrate this the results of the blood examination of a few animals, all first relapse cases exhibiting the most irregular infections that were observed, are given below (Table III) The first positive finding after treatment is given as R, 4731 and 4738 were infected with *T congolense*, the other with the Emali strain of *T vivax*

(iii) It will be agreed that, with the possible exception of some strains of *T vivax*, trypanosomes appear in the blood with sufficient regularity for a relapse to be easily discerned, provided that smears are read regularly and conscientiously It was because of this last proviso, coupled with the importance of the experiments as a whole, that the wearisome task of reading the many smears involved was done for the greatest part by reasonably skilled and responsible people All the smears, except those of the curative experiment with *T congolense* in Uganda using the chloride, were read by my technicians or by myself The smears of the Uganda experiment were read by African assistants of the Entebbe laboratory under the supervision of Mr S G LAWS

(iv) It would seem that, in experiments such as these, regular examination of smears over a long period is to be preferred for the diagnosis of trypanosomiasis rather than occasional subinoculation because, contrary to what is sometimes supposed, the circulating blood even in infections with *T congolense* may be apparently completely free of trypanosomes Thus, towards the end of the curative experiments in the Sudan, a pooled subinoculation was made from a group of apparently negative animals The recipient animal showed trypanosomes, whereupon each of the donor animals was subinoculated individually—with completely negative results Subsequently, one of the donor animals relapsed and so explained the first positive subinoculation In other words, although there may clearly be occasions when trypanosomes in the circulating blood are so scanty that they cannot be observed in smears with certainty, there may also be occasions when they may be so scanty if, indeed, they are present at all in the circulating blood, when subinoculation does not reveal their presence

My own view is that trypanosomes are present in the fluids of the tissue spaces as well as in the blood (this is very easily shown to be true in the small laboratory animals) and that during the apparent negative phase of the circulating blood before a relapse occurs, the blood may actually be free of trypanosomes and the infection be surviving in the tissue spaces. This is at least the most rational explanation of the following experiments.

TABLE III. Course of first relapse infections of *T. congolense* and *T. vivax* in cattle.

Number	Days after treatment.											
	21	22	23	24	25	26	27	28	29	30	31	32
Congolense												
4731	R	1/5	1/2	1/"	1/5	—	—	—	—	—	—	—
4738	—	—	—	—	—	—	—	—	—	—	—	R
	40	41	42	43	44	45	46	47	48	49	50	51
Vivax												
2476	R	1/8	1/5	—	—	—	1/40	1/8	1/1	1/20	1/20	1/20
5116								R	—	1/100	—	—
5440			R	8/1	20/1	—			—	—	—	—

Number	Days after treatment.											
	33	34	35	36	37	38	39	40	41	42	43	44
Congolense												
4731	1 20	1 2	1 8	2 1	5 1	8/1	1/20	—	—	—	—	—
4735	1 5	1 5	1 3	1 1	1 2	—	—	—	—	—	—	—
	52	53	54	55	56	57	58	59	60	61	62	
Vivax												
2476	1 ?0	1 50	1/100	1 5	—	1 10						
5116	1 50	—	—	—	1 20	1 5	1/60	1/20	1/50			
5440	—	1 100	1 100	—	—	—	—	—	—	—	—	

Number	Days after treatment.										
	45	46	47	48	49	50	51	52	53	54	55
Congolense											
4721	1 100	—	1 1	1 10	1 10	—	—	—	1 50	1 30	
4735	—	—	—	—	—	—	1 50	1/8		1/1	

Six mice infected with *T congolense* (Busimbi strain) were treated with a dose of antrycide chloride (005 mg per 20 gramme mouse s c) known to give relapses On the second, fourth and seventh days after treatment 0 3 to 0 4 c c of blood, drawn from the tail vein, was subinoculated from each mouse into clean mice Four of the six recipients receiving blood on the second day became infected, none on the fourth day, and three on the seventh day Patent relapses started in the original group on the ninth day

In a second experiment, mice treated in the same way were killed and as much blood as possible collected from each mouse and injected into two clean mice Recipients injected on the fourth and sixth days after treatment did not become infected, those injected on the seventh day did

(v) For how long after treatment should smears be taken? In these experiments the period of 16 weeks (112 days) was only decided upon after the first curative experiment in the Sudan had been running some considerable time It appeared that the majority of relapses occurred within about 2 months after treatment and that only odd ones occurred after that time Consequently, it seemed reasonable to suppose that the great majority of animals which appeared negative to all examinations over a period of 16 weeks were actually cured The distribution of relapses over time is shown in Table IV, in which the relapses from all the doses and all the experiments with *T congolense* are collected together Results with antrycide chloride and with antrycide methylsulphate are kept separate because the better prophylactic effect of the former possibly tends to lengthen the period to relapse Relapses observed with *T vivax* all took place within less than 8 weeks after treatment

TABLE IV Time-distribution of relapses with *T congolense*

Salt.	Number of relapses	Distribution—weeks after treatment													
		3	4	5	6	7	8	9	10	11	12	13	14	15	16
DiCl	41	16	2 18	4 22	3 25	2 27	3 30	2 32	2 34	2 36		3 39	1	1	
DMS	14	2	3 5	4 9	2 11	1 12	2 14								

Curative Experiments with T congolense

The results of the curative experiments with *T congolense* are summarized below in Table V All the cattle were treated subcutaneously

Comments

(i) All weights of methylsulphate should be multiplied by 1 32 to give the actual weight of drug

(ii) Particulars of the strains used in these experiments are as follow

The Southern Sudan strain was collected from naturally infected bovine brought into Malakal. Blood from this beast was injected into bull which served as donor for the experimental group.

The M bende strain was collected from cattle found naturally infected in the Mubende district Uganda. Blood from these cattle was used to infect mice on the spot, and some was drawn int citrat -saline The citrated blood was injected 6 to 8 hours later int the cattle which were subsequently treated with antrycide chloride. Blood from the mice was used to infect the cattle treated with the methylsulphate

The strain designated Kenya T.90 is an old laboratory strain which has also been used in experiments by Dr S F BARNETT and by Mr R N T W FIENNES. It has been passaged mechanically for several years in cattle and in mice, and has also been exposed to dimidium bromide The experimental cattle were infected by the injection of blood from mice.

The Mariakani strain was collected at Mariakani, near Mombasa, in Kenya, but probably came from Northern Kenya. It was maintained by Dr E. A. LEWIS by passage through G *austeni* The experimental cattle were infected by the bite of tsetse flies.

TABLE V Curative results with T congolense [illegible] cattle.

Dose mg. per kg s.c.	Sudan (strain Southern Sudan).		Uganda (strain Mubende).		Kenya I (strain Kenya T 90).		Kenya II (strain Mariakani).
	DrCl.	DMS.	DrCl	DMS.	DrCl.	DMS.	DMS.
0 1	3 3R						
0 25	4 4R	1 1R	3 3C	1 3R 3C			
0 5	R		3 12R 9 12C	4 4C	8 8R	5 5R	1 3R 3C‡
1	3 7R† 7C	3 3C 2 2C (prob)	1 1 R 11 12C	8 8C	6 6R	5 6R 1 6C	9 9C‡
	1 7R 6 C		12 12C		5 12R 7 12C	1 11R 10 11C	
4	3 3C				6 6C		
1 DMS DrCl					4C		

DMS antrycide methylsulphate DrCl antrycide chloride C cure R relapse

three of three animals relapsed

† i.e. five of seven animals relapsed, two of seven were cured

‡ probable cures.

(iii) All these strains were virulent in the sense that they readily infected cattle, and sooner or later killed cattle not treated

(iv) A study of Table V shows that antrycide can be used to cure infections of *T congolense* in cattle, that the methylsulphate is superior to the chloride for this purpose, and that there is a variation between strains in their susceptibility to the drug, the Mubende strain being the most and the Kenya T 90 strain the least susceptible It has been suggested that the numbers of animals used are too few for conclusions to be drawn regarding a curative dose, but this seems to me to be true only if we are seeking the smallest possible dose that will cure all animals, and we are not On the contrary, we are seeking a dose which is sufficiently high to allow a margin of error in treatment The dose that has been chosen is 5 mg per kg of methylsulphate (equivalent to about 4 mg per kg DMS as the figures are calculated in the table), and the question to be answered is Will this dose cure infections of *T congolense* and allow a margin of error? (We are assuming, of course, that the four strains are fairly representative of the species) If each strain is considered separately the answer is clearly yes, in all probability

(v) For interest, three of the strains of *T congolense* tested against antrycide were also tested against dimidium bromide using the usually recommended field dose of 1 mg per kg The drug was given subcutaneously With the Sudan strain two of three animals relapsed, with the Kenya T 90 strain two of four relapsed, and with the Mubende strain six of six were apparently cured

Curative Experiments with T vivax

The results of these experiments are summarized in Table VI The cattle were treated subcutaneously

TABLE VI Curative results with *T vivax*

Dose mg per kg s c.	Kenya (strain Emali)		Uganda (strain Musoli forest)	
	DiCl	DMS	DiCl	DMS
1		1/6R 5/6C	1/1R	1/2R 1/2 ?
5	5/5C	5/5C		6/6C*
10				2/2C*
1 DMS + 2 DiCl	6/6C			
1 DMS + 5 DiCl	2/2C			

* See comment (iia) on page 594

Comments

(i) The Emali strain, which was collected by Dr E. A. Lewis at Emali in Kenya, has been maintained by him in *G. pallidipes*, and all the animals used in these experiments were infected by the bite of infected flies. The strain is noteworthy as was mentioned above for its virulence which makes it most convenient for tests such as these.

(ii) The Musoli Forest strain was collected from laboratory cattle which had grazed near the edges of the forest. Some of the forms were short and, according to Dr S. G. Wilson some *T. uniforme* was probably mixed with *T. vivax*. Whether this is important or not cannot be judged, because *T. uniforme* appears to resemble *T. vivax* in most respects other than length. The value of the experiments done with it is doubtful because the virulence of the strain and so the general "readableness" of the course of infection is uncertain. The relevant facts are as follow

(*a*) A group of nine animals were infected by the intravenous injection of whole blood (each animal received 100 c.c.) drawn from a naturally infected bovine. Five of them were showing trypanosomes 8 days later two others showed *trypanosomes the next day and an eighth showed trypanosomes by the* 11th day after injection. These eight animals were treated on the 12th day and are the animals asterisked in the summary of the curative results with *T. vivax* given in Table VI

(*b*) The ninth animal (No. 78) did not show trypanosomes until 18 days after infection. Its subsequent history is given in Table VII

TABLE VII. Course of infection of *T. vivax* Musoli forest strain in bovine 78.

Number	Days after infection. Parasite density in the blood on the indicated day after infection.															
78	16	1	1	19	20	21	22		39		41	45	46	47	48	49
			1:10	1:5	1	1:1	—	Negative during this period	1	Negative during this period	1:1		—	1:100	1:5	

It was negative thereafter until the 150th day when examinations ceased.

(c) On the 38th day after infection Bovine No. 78 was subinoculated to challenge a group of cattle used in the Entebbe prophylactic experiments. Each animal received 40 c.c. whole blood intravenously Two controls (107 and 4926) were used the course of infection in these animals is given in Table VIII

TABLE VIII Course of infection of *T vivax* Musoli forest strain in bovines 107 and 4926

Number	Parasite density in the blood on the day indicated after infection									
	4	5	6	7	8	9	10	11	12	13
107	—	—	1/5	5/1	10/1	1/10	1/2	2/1	5/1	1/5
4926	—	—	1/5	1/5	1/5	2/1	2/1	1/1	1/5	1/10
Number	**Parasite density in the blood on the day indicated after infection**									
	14	15	16	17	18	19	20	21	22	23
107	1/2	10/1		2/1		1/5	5/1	—	—	1/50
4926	2/1	1/2	1/100	—		1/2	2/1	2/1	1/50	1/10
Number	**Parasite density in the blood on the day indicated after infection**									
	24	25	26	27	28	29	30	31	32	33
107	1/5		Infected *T congolense*	—		—		5/1	1/10	congolense showing and vivax
4926	Treated									

There is clearly nothing remarkable about the behaviour of the strain in Bovines 107 and 4926, and one would not hesitate to say from these results that the strain could be used in curative experiments

The behaviour of the strain in Bovine No 76—witness the long prepatent period and the infrequency with which trypanosomes appeared in the blood —may not be typical, therefore, but it has to be taken into account in assessing the value of the experiment as a whole

(iii) For reasons already explained, information on *T vivax* is not as complete as would be desirable, and all that can be claimed is that the recommended field dose of 5 mg per kg antrycide methylsulphate has been used to cure one virulent strain of *T vivax* and has probably cured another, the general pathogenicity of which is uncertain

PROPHYLACTIC EXPERIMENTS

The course followed in the prophylactic experiments done in cattle in the laboratories at Khartoum, Entebbe and Kabete was controlled entirely by the process of feeling one's way knowing, because of the duration of the experiments,

that there would be no opportunity of retracing one's steps. The first stage that of confirming the fact that the prophylactic properties of antrycide exhibited in small laboratory animals would also be exhibited in cattle (and other animals), was done in Khartoum. Two doses were chosen, the first, 1 mg per kg antrycide chloride, or what might be a minimum curative dose for *T congolense* (the experiments were started within a week of the commencement of the actual curative experiments) and the second 5 mg per kg antrycide chloride or what might be the largest dose necessary for the cure of *T congolense* Then, when it became clear that 1 mg per kg chloride could not be regarded as a curative treatment, the treatment for the prophylactic groups at Kabete was altered to 2 mg per kg chloride and 5 mg. per kg chloride. At Entebbe two groups of cattle were treated with bulk doses of gramme 1 and gramme 2 chloride respectively because it was thought that bulk doses, rather than doses calibrated in mg per kg., might be more convenient for use in the field.

Throughout this time supplies of the methylsulphate were restricted, and it was only after the experiments with the chloride had been in progress for some months that comparative experiments with the methylsulphate were started. Later still it became clear that neither the methylsulphate no the chloride would be used alone for prophylaxis, and so attempts were made, particularly in the field experiment at Kiboko to obtain information on the effect of a mixture.

The process of feeling one's way also governed the duration of the period allowed to elapse between treatment with the drug and attempted infection. After short periods of time (2 to 3 months or less) nly one or two animal of a group would be exposed to infection (*i.e.*, challenged) most being kept back until the indicator cattle had given results.

Most of the cattle were exposed to infection by the injection of infected blood either intravenously or subcutaneously (syringe-challenge). One would have liked more to be exposed to the bite of infected tsetse flies, but except in Kenya our facilities were not adequate for this to be done. " Syringe challenge was therefore resorted to because the results would be of importance in any case, if mechanical transmission is also of importance in the field, and because there is no reason to suppose that the difference between the results achieved by " syringe-challenge " and by " fly-challenge should be remarkably different. Thus in malaria we know that the stages in the life cycle of the parasite represented by sporozoites and exo-erythrocytic forms react very differently to drugs from the stages within the red blood corpuscles but there is no evidence that a succession of stages exists in the life history of trypanosomes in the vertebrate host (except, of course, in the case of *T cruzi*). Again, drugs such as suramin, which have a marked protective action against syringe challenges in laboratory animals hav a prophylactic action in the field, and others, such as dimidium bromide, which exert little protective action in laboratory animals, exert little n the field.

As the experiments progressed confirmation was obtained that the results achieved by "syringe-challenge" were not very different from those obtained by "fly-challenge" In the first place, cattle maintained in tsetse areas were protected by antrycide for about as long as the experiments with "syringe-challenges" suggested that they might be In the second place, a direct comparison was made between "fly" and "syringe" challenges through the generous co-operation of Dr E A LEWIS, of the Kenya Veterinary Department Dr LEWIS has built up a unit for handling tsetse, and it was only through his generosity in placing the facilities of his unit at the disposal of these experiments that the fly infections and fly challenges recorded in this work were made possible

The comparison between "fly" and "syringe" challenge was made on a group of cattle, each of which had been treated with gramme 1 5 antrycide methylsulphate together with gramme 1 antrycide chloride The time of challenge was so chosen that it was hoped some infections would result, although actually only one animal became infected The comparison could not be made absolutely strictly because there is no way of telling how many trypanosomes a tsetse fly injects when it feeds The challenges were therefore made as heavily as possible The results are given in Table IX One would have liked the

TABLE IX. Comparison between "syringe" and "fly" challenge

[Each animal had been treated with gramme 1 5 DMS + gramme 1 DiCl, challenges were made 14 weeks 6 days after treatment]

Group I—"Syringe" challenge. Each animal received 20 c c. heavily infected blood subcutaneously, the prepatent period in six controls varied from 7 to 10 days				Group II—"Fly" challenge. Each animal was exposed to the bite of one infected fly on the first day, and to a group on the next day The number which fed is given below				
Number	Weight		Result	Number	Weight		Number of flies which fed	Result
	At treatment	At challenge			At treatment	At challenge		
5901	205	255	−42*	5917	206	192	1 6	−42
5903	215	254	−42	5918	180	215	1 7	−42
5905	212	266	−42	5919	157	226	1 6	−42
5908	186	227	−42	5920	191	228	0 5	−42
5909	173	224	−42	5921	209	278	1 6	−42
5912	140	181	−42	5922	162	198	0 5	−42
5915	170	202	−42	5923	193	258	1 6	−42
5916	183	240	−28D	5929	136	194	1 5	+ 9

* i e, negative to successive daily examinations of thick blood smears for 42 days after challenge

examination of the exposed cattle to have been carried on for a longer time, but this was not possible. However it is clear that there is no indication from the results that a significant difference exists in the protection afforded by antrycide towards "fly" and "syringe" challenges.

The results of the prophylactic experiments with the chloride are summarized in Table X. The results obtained with the methylsulphate have not been summarised in tabular form since it is sufficient to say that whereas all animals treated with 5 mg. per kg. chloride resisted infection when challenged with *T. congolense* at 12 weeks, and the majority resisted infection when challenged at 24 weeks, all except possibly one animal treated with 5 mg. per kg. methylsulphate became infected when challenged at 12 weeks.

The summary in Table X requires some explanation. To make presentation easier times have been approximated and some animals have been included more than once. For example, challenges made at about 19 weeks have been included in the column of results at 20 weeks, and when a group of animals have received both *T. congolense* and *T. vivax* the results are recorded separately. The letters K, S and U indicate that the experiments were done in Kenya (Kabete), the Sudan (Khartoum) or Uganda (Entebbe) and if followed by the letter T it means that the group of cattle had been used in therapeutic experiments and that they were then challenged with the homologous strain. The ratios and the signs give the number of animals challenged and the results for example, C 1/3 – 2/3 + means that three animals were challenged with *T. congolense*, one resisted infection and two did not. The vivax fly challenges (indicated by K^{fv}) were made with *G. palpalis* infected with the Emali strain: usually six or more infected flies were used and they were either fed all at the same time, or in groups of two or three every day or every other day on three or four occasions. Where a result is questioned either there is an element of doubt in the reading of a smear or observations could not be continued sufficiently long to justify a conclusion. Actually it is still problematical how long an animal should be observed after trypanosomes are injected before the statement can be made that it has resisted infection. In these experiments it was considered that daily examinations of thick blood smears for 84 days should be sufficient to warrant a conclusion being drawn—some animals were observed for an even longer time—but sometimes, for one reason or another it was not possible to do this. The question therefore arises: What is the minimum time an animal should be observed before some conclusion can be reached? An approximate answer can be obtained from a consideration of the distribution in time of those "break throughs" that were observed. In the experiments with *T. congolense* and *T. vivax* no unequivocal "break through" was recorded at any time longer than 35 days after challenge: one was recorded at this time, and all the others occurred at times spread out between the prepatent period in untreated animals (usually 5 to 10 days or so depending on the inoculum) and 31 days. It would appear likely, therefore

TABLE X. Summary of the results of prophylactic experiments with antrycide chloride in cattle

Dose mg per kg	Challenges Period after treatment and results						
	4 weeks	8 weeks	12 weeks	16 weeks	20 weeks	24 weeks	30 weeks
1	C 1/1− (S)	C 1/1− (S)		C 1/3−, 2/3+ (UT)			
2	C 1/1− (K)			C 3/7−, 4/7+ (KT) C 2/2− (K) C 3/6−, 1/6?, 2/6+ (UT) V 5/6−, 1/6?	C 1/1− (K$^{\mathrm{fly}}$)	C 3/6−, 3/6+ (K) V 1/3−, 2/3+ (K$^{\mathrm{fly}}$) V 3/3+ (K)	
2+ 1 DMS				C 3/3− (K$^{\mathrm{fly}}$)	C 3/3− (K$^{\mathrm{fly}}$)		
4					V 1/6−, 5/6+ (K$^{\mathrm{fly}}$)		
5	C 1/1− (S) C 1/1− (K)	C 1/1− (S)	C 1/1− (S) C 2/2− (K) C 2/2− (U)	C 2/2− (K)	C 1/1− (K) V 1/1− (K$^{\mathrm{fly}}$) V 5/5− (U)	C 3/7−, 1/7?, 3/7+ (K) C 3/5−, 1/5?, 1/5+ (U) V 2/3−, 1/3+ (K$^{\mathrm{fly}}$) V 2/3−, 1/3+ (K)	C 4/5−, 1/5? (S)
6–8					V 2/3−, 1/3? (U)	C 2/3−, 1/3? (U)	
9–11					V 5/5− (U)	C 5/5− (U)	C 8/9−, 1/9? (S)

C = *T congolense*, V = *T vivax*, S = Sudan (Khartoum) experiment, K = Kenya (Kabete) experiment, U = Uganda (Entebbe) experiment, T = cattle used in therapeutic experiment, cured and challenged with homologous strain, fly = fly challenge, the negative sign indicates that infection was resisted so C 1/3−, 2/3+ means 1 of 3 animals challenged with *T congolense* resisted infection, and 2 of 3 became infected

majority of animals which yield negative daily blood smears for about *after challenge have actually resisted infection.* The prepatent periods *observed in animals challenged during these experiments,* and which nfected, are given in Table XI

XI Prepatent period of break-through infection recorded in these experiments.

...es.	Normal prepatent period (days).	Prepatent periods in days of break-through infections.
...elense	5–10	10 11 11 14 16 18 19 24 26 27 28, 29 31 33
	5–10	10 11 14 18 19 1 21 26 28 29 31

prophylactic experiments taken as a whole are of value in showing yoide chloride possesses marked prophylactic properties that the lphate is inferior to the chloride in this respect and that, in future lt is not possible to work with tsetse-borne infections, valuable infor an still be obtained using trypanosomes transmitted mechanically import of the results from a practical point of view will be considered

THE FIELD EXPERIMENTS.

field experiments were done one at Nsongezi near Mbarara in which started *in June, 1948* the other at Kiboko *about 100 miles robi on the* Nairobi-Mombasa Road, which started in October 1948. ngezi area was suggested by Mr A CROWLEY Director of Veterinary Uganda, and the Kiboko area by Dr E. A LEWIS, Chief Field , Kenya Veterinary Services. The Nsongezi area is infested by *ans* the Kiboko area mainly by *G pallidipes* and *G brevipalpis* together *e G longipennis.*

setting up of an experiment in a tsetse area involves quite consider ier A boma has to be built to protect the cattle at night against very necessary precaution at both Nsongezi and Kiboko—and guards dsmen have to be employed and supervised. The experiments at would not have been possible without the *considerable help of* Mr itts and Mr R. N SANDERS, of the Uganda Veterinary Department, at Kiboko could not have been carried out without the co-operation L. N. T W FIENNES and Dr E. A. LEWIS and his staff

en the Nsongezi experiment started it was *still uncertain which salt* ide might be used in the field, and a straightforward *comparison* was tween the methylsulphate and the chloride. Ten cattle were each

eated with gramme 2 of the former and 10 with gramme 1 5 of the chloride he two amounts are roughly equivalent in terms of antrycide proper)

By the time the Kiboko experiment was started it was known that the chloride would not be issued alone, and so, still seeking a treatment which might be recommended for use in the field, the methylsulphate was tried alone in a big dose (gramme 3 to each beast), and mixed with the chloride The mixtures contained a curative dose of the methylsulphate (gramme 1 5), together with varying amounts of chloride (gramme 0 5, gramme 1, and gramme 2)

The results of the two field experiments are summarized in Tables XII and XIII In the summaries the results are recorded additively, that is to say, an animal in which trypanosomes have been observed is thereafter always regarded as positive irrespective of later readings although, of course, in practice the infections of *T vivax* wax and wane

Thick blood smears were taken at Nsongezi on two successive days about every fortnight, at Kiboko they were taken on two successive days each week for the first few months, and later three times a week at spaced intervals

TABLE XII The experiment at Nsongezi

Group	Results—weeks after treatment.											
	7	9	11	12	14	16	18	20	22	25	27	28
I Each animal received gramme 2 DMS	10—	10—	7— 3V	3— 7V	3— 7V	1— 9V	1— 9V	1— 8V 1 dead	1— 8V	1— 6V 2 dead (3)	1— 4V 2 dead (5)	1— 3V 1 dead (6)
II Each animal received gramme 1 5 DiCl	10—	10—	10—	9— 1?	7— 2? 1V	5— 1? 4V	3— 7V	3— 5V 1VC *1K	3— 5V 1VC	3— 5V 1VC	3— 5V 1VC	3— 5V 1 dead (VC)

* One killed by lion C = congolense, V = vivax.

Comments on the Experiment at Nsongezi

(i) Five controls were introduced into the fly area at the same time as the treated cattle The first died 5 weeks after entry showing *T vivax* only, the four others became infected with both *T congolense* and *T vivax* and all died within less than 12 weeks after entry

(ii) If the one control which died showing *T vivax* only is a true indication then the strain of *T vivax* in the area may be fairly described as virulent It is of interest, therefore, that many of the treated animals were able to carry an infection with the strain for a surprisingly long time without exhibiting

obvious symptoms of ill-being. This fits in with other observations made during the course of all the experiments reported here. It seems that if the course of infection with a strain of *T. vivax* that normally is highly virulent (for example, the Emali strain) is checked or impeded in any way animals are able to develop a tolerance towards it and carry it easily for a long time.

(iii) The chloride is clearly superior to the methylsulphate in prophylactic properties.

(iv) Although the controls demonstrated that *T. congolense* was in the

TABLE XIII. The

Treatment.	Number in group	Results—Weeks								
		9	10	11	12	13	14	15	16	17
Gramme 1·5 DMS + gramme 0·5 DaCl	1	1 —	12—	1 —	1 —	8— 3N 1P	8— 3N 1C	8— 3N 1C	8— 4N 1NC 1 dead (V)	(11) 5— 4N 2NC
Gramme 1·5 DMS + gramme 1 DaCl	11	11—	11—	11—	11—	11—	9— 1N 1T	7— 2N 2T	7— 1N 2T 1 dead (V)	(10) 7— 1N 2T
Gramme 1·5 DMS + gramme 2 DaCl	11	11—	11—	11—	11—	10— 1T	10— 1T	10— 1T	10— 1T	10— 1T
Gramme 3 DMS	1	9— 1N	8— 1T 1 dead (V)	(9) 8— 1N	7— 1N 1T	6— 1N 2T	3— 3N 1T	4— 4N 1T	4— 4N 1T	3— 3N 1C 1NC 1T

1.—(i) The number in parentheses gives the number of animals left alive in the group at that particular time.

area, only one of the treated animals—and this one, oddly enough, received chloride—became infected This might be explained if the infection rate of the tsetse fly at Nsongezi with *T congolense* is low, because the impression has been obtained from other experiments that an animal treated with antrycide resists a "light challenge" better than a "heavy" one

(v) A group of animals not included in the summary which had been treated with gramme 2 antrycide chloride on 11th March, 1948, were introduced into the fly area on 29th July, 1948, almost 5 months after treatment

...xperiment at Kiboko

...after treatment.

18	19	20	21	22	23	24	25	26
5— 4V 2VC	3— 4V 2VC 2 dead (—) 1 dead (V)	(8) 3— 2V 2VC (1VC treated)	(7) 3— 2V 2VC	2— 2V 2VC 1 dead (VC)	(6) 2— 1V 2VC 1 dead (VC)	(5) 2— 1V 2VC	2—	2—
7— 2V 1T	6— 3V 1T	6— 3V 1T	3— 4V 1C 1 dead (—) 1 dead (V)	(8) 2— 5V 1C	2— 3V 1C 1 dead (V) 1 dead (VC)	(6) 2— 3V 1C	2— 1C	
10— 1T	8— 3V	7— 3V 1C	5— 4V 1C 1 dead (—)	(10) 5— 4V 1VC	4— 4V 2VC	4— 4V 2VC	4— 4V 2VC	
3— 4V 1C 1VC	2— 4V 2C 1VC	2— 4V 2C 1 dead (VC)	(8) 2— 3V 2C 1 dead (V)	(7) 2— 3V 2C	2— 3V 2C	2— 3V 2C	1— 4V 2C	

(ii) V = vivax, C = congolense, T = trypanosome, species not identified

One of them, apparently still free of trypanosomes, was killed by lion 13 weeks later The four others gradually became infected with *T vivax* but they were all alive, and reasonably fit, at the beginning of January 1949 Two controls introduced into the area at approximately the same time quickly became infected and would have almost certainly died if they had not been treated.

Comments on the Experiment at Kiboko

(i) The area was heavily infested with tsetse fly and the incidence of infection of the fly with trypanosomes may be supposed to be high since untreated cattle taken to the area quickly became infected with *T congolense* *T vivax* and *T brucei* Of six controls to the experiment, none survived longer than about 9 weeks.

(ii) The summary in Table XIII will show at a glance the position of the experiment at any particular time after treatment (*T brucei* has been omitted from the summary since this species does not seem to be of importance in cattle).

(iii) The most protection was afforded by *gramme 1 5 methylsulphate* mixed with gramme 2 chloride that afforded by gramme 1·5 methylsulphate mixed with gramme 1 chloride was also good.

(iv) In the group receiving gramme 1 5 methylsulphate mixed with gramme 1 chloride, *T vivax* was first found in blood smears in any animal 14 weeks after treatment, and *T congolense* 21 weeks after treatment. In the group receiving gramme 1·5 methylsulphate mixed with gramme 2 chloride, *T vivax* was first found in any animal 13 or 19 weeks after treatment (the two trypanosomes found in the blood smear taken at 13 weeks could not be definitely identified as either *T vivax* or *T brucei*), and *T congolense* 20 weeks after treatment. Only two animals in each group became infected with *T congolense* although they were examined for approximately 26 weeks after treatment, of which about 24 weeks were spent in the tsetse area.

(v) Deaths amongst the treated animals cannot be accounted for satisfactorily Two infected with *T vivax* were diagnosed by Mr R. N T W Fiennes after postmortem as having died from trypanosomiasis, but of the others all that can be said is that four died without even showing trypanosomes in blood smears two died infected with *T congolense* and *T vivax* and probably did die as a result of their infection and five died showing *T vivax*. (Piroplasmosis possibly contributed to the death of one of the latter) Looking back over this experiment, and the one at Nsongezi, it is clear that t obtain complete and accurate information concerning all occurrences it is necessary that a skilled and responsible person should be continually present. It is fair to say that the cattle in the two field experiments considered in this report were guarded but were hardly cared for

(vi) The practical implications of the results will be considered later

RESISTANCE

It can be taken almost as axiomatic that trypanosomes are able to develop resistance towards any drug exerting an action on them, and it would have been foolish to have supposed that antrycide would have been an exception On the contrary, its persistence in the body, the very property which confers on it the power of prophylaxis, favours the creation of resistant strains of trypanosomes should the drug be mishandled The development of resistance to antrycide was studied in curative experiments and in prophylactic experiments

1 *The Resistance of Relapse Strains*

The animals that relapsed in the first experiment with *T congolense* in the Sudan (Table I) were re-treated with the results shown in Table XIV

TABLE XIV Re-treatment of *T congolense* relapses

Number	First treatment		Patency (days)	Second treatment		Patency (days)	Third treatment	
	Mg per kg	Result		Mg per kg	Result		Mg per kg	Result
804	0 1 DiCl	R^{14}	18*	1 DiCl	R^{23}	8	4 DiCl	R
890	0 1	R^{12}	4	1	R^{31}	16	4 „	R
915	0 1	R^{7}	0	1 „	R^{15}	2	2	R
845	0 25 „	R^{20}	42	4	R			
847	0 25	R^{20}	12	1	R	10	4	C?†
892	0 25	R^{14}	18	2 „	R			
905	0 25	R^{64}	8	4 ,	R			
851	0 5 „	R^{90}	12	1 DMS	C			
860	0 5	R^{56}	6	4 DiCl	R			
894	0 5	R^{23}	6	1	R	9	4	C?†
907	0 5 „	R^{30}	2	2	$—^{61}$			
840	0 5	R^{42}	15	4	R			
891	0 5 „	R^{43}	16	4	R			
903	0 5 „	R^{17}	12	1 „	R	14	4 ,	R
896	1	R^{35}	24	4	R			
909	1	R^{60}	2	4	R			
848	1	R^{61}	13	4	C?†			
893	1	R^{55}	4	4 „	R			
906	1	R^{17}	12	2	C?†			
908	2	R^{88}	10	1 DMS	C			

C = cure , R = relapse

* This period (patency in days) is the period during which the infection was patent before the next treatment was made

† The cure is questioned because observations were made only irregularly

Comments on Table XII.—(i) If we suppose that 2 mg. per kg. antrycide chloride should have cured most of the animals, which is a fair supposition from the results already given in Table I, and that 4 mg. per kg. should have cured all, then it is clear that even a first relapse strain after chloride treatment has acquired a good measure of resistance.

(ii) How much significance should be placed on the fact that two relapses treated with 1 mg. per kg. DMS were both cured (numbers 851 and 908) is uncertain because both happened to be very late relapses, and whether there is a difference between an early and a late relapse in its response to treatment is not known. It is true, however, that a better result would be expected following treatment with the methylsulphate because of its better absorption.

(iii) The results given in this table form one of the main reasons why it is recommended that the chloride should not be used alone. It is wisest to assume that all relapse strains are potentially resistant, and therefore relapses should be avoided. In other words, a curative treatment should err on the generous side, and it is easier to arrange this with the methylsulphate. SPINKS (private communication) has shown that the absorption of this salt proceeds stepwise as the dose is increased, and concentrations in the blood are almost directly proportional to the size of the dose. With the chloride, on the other hand, this is not so, and there appears to be very little measurable difference between peak concentrations at any particular dose and at twice that dose, although there will be a difference in the persistence times. It follows that a relapse strain being re-treated with chloride is being exposed to peak concentrations very little different from those which it has survived and thus, of course, will strengthen its resistance.

2. *The resistance of "break-through" strains following prophylaxis*

When a drug is given to confer protection to an animal against infection with trypanosomes there comes a time when the concentration falls below the level necessary for complete protection. If trypanosomes are introduced into the body at this time and survive or break through this dwindling concentration, will they become trained to resist higher concentrations? The question is clearly applicable generally to the employment of drugs for prophylaxis against trypanosomiasis, but as far as I am aware it has been investigated only for antrycide.

The investigation took three lines. Firstly, infections that occurred during the normal run of the prophylactic experiments were tested for susceptibility. Secondly, break-through infections were deliberately sought for in cattle repeatedly challenged with *T. vivax* (Emali strain) transmitted by *G. pallidipes*. Thirdly, breaks through of *T. congolense* at Kiboko were subinoculated into mice or rats and then tested for susceptibility to antrycide.

(i) *The susceptibility of break-through strains that appeared during the prophylactic experiments*

The details of the animals on prophylactic experiment which became infected when "challenged" and so served for the purpose of investigating the susceptibility of the break-through strains to further treatment with antrycide, are given in Table XV Some idea of the difficulties encountered by the trypanosomes in becoming established in the cattle is reflected in the prepatent period of the break-through infection Where this approximates closely to the prepatent period in controls, the infection hardly merits the description "break through," for clearly there can have been little drug to obstruct the trypanosomes Where, however, it is much longer, say of the order of 20 days or more, it can be taken that the success of the trypanosomes in becoming established was finely weighed against failure

The facts presented in Table XV are not sufficient for one to say that these particular break-through strains had no resistance towards antrycide, but they are sufficient for one to say that the degree of resistance was not so great that antrycide could not be used to eradicate them Thus the minimum curative dose of antrycide methylsulphate for the Kenya T 90 strain of *T congolense* is about 2 mg per kg, and six of seven good examples of breaks through were cured with 3 mg per kg, two other good examples (in bovines number 3046 and 4725) were cured with 5 mg per kg Again, the minimum curative dose of the *T vivax* Emali strain is 1 to 2 mg per kg antrycide methylsulphate and three good examples of break-through strains were cured with 2 mg per kg, and six with 5 mg per kg

(ii) *The susceptibility of break-through strains of* T vivax *(Emali strain) obtained by repeatedly challenging treated cattle with infected tsetse flies*

In these experiments cattle which had not previously been infected with *T vivax* were given 1 mg per kg antrycide chloride, *i e*, an amount which would not protect so long as to be inconvenient, and then repeatedly exposed to the bite of *G pallidipes* infected with *T vivax* (Emali strain) until they became infected Other cattle known to be protected by antrycide were similarly challenged until they also became infected

It was hoped that by repeatedly challenging in this way the earliest possible break-through strains would be obtained In other words, it was hoped that trypanosomes which had broken through the highest concentration of drug they were capable of surviving would be obtained for their susceptibility towards antrycide to be tested

This sort of experiment demands the services of a very efficient unit for raising and infecting tsetse flies, and again I have to thank Dr E A Lewis for his generous help Many of the cattle did not exhibit trypanosomes in

TABL

Group	Number	History	Treatment through which break through appeared (mg. per kg.)	Species and strain
A. *T. congolense* breaks through	321	Cured T.V. Emali, then this challenge	5 DMS	TC Kenya T 90
	4703	Challenged at 16W (C) then this challenge	3 DzCl	TC Kenya T 90 TV Emali
	8341	Sub. from 4703	—	—
	4742	Cured TC Kenya T 90, then this challenge	2 DzCl	TC Kenya T 90
	4746		2	
	5025		2	
	5196	Cured T.V. Emali, then this challenge	5 DMS	
	5198		5	
	5046	Cured T.V. Emali, then this challenge	5 DMS	TC Kenya T 90
	5036	TC Kenya T 90 then this challenge		
	5035	Cured TC Kenya T 90 then this challenge	DMS	TC Kenya T.90
	5037			
B. *T. vivax* breaks through	5033	Cured T.V. Emali, then this challenge	DzCl 1 DMS	TV Emali fly
	4723	Clean until this challenge	5 DzCl	TC Kenya T 90 TV Emali fly
	9931	Sub. from 4723	—	—
	5341	Clean until this challenge	5 DMS	TV Emali fly
	4748	Cured TC Kenya T 90 then this challenge	4 DzCl	TV Emali fly
	4749		4	
	4750		4	
	475_		4	
	4753		4	
C. Mixed *T. congolense* and *T. vivax* breaks through	4695	Clean until this challenge	2 DzCl	TC Kenya T 90 TV Emali
	4701		2	As above
	4696	Challenged at 16W (C), then this challenge	[illegible]	TC Kenya T 90 TV Emali
	4725	Clean until this challenge	[illegible]	As above

... one trypanosome found at 18 days and infectu

Period after treatment.	Break-through period after challenge (days)	Prepatent period in controls (days)	Treatment of break through		Result.
			Weight (kg)	Dose mg per kg	
12 weeks	18—31*	9	300	3 DMS	—^{113}
25 weeks 1 day	C+19 V—	14	142	3 ,,	—^{106}
—	—	—	232	3 ,,	R^{64}
17 weeks	26	8	330	3 ,,	—^{30}D†
17 , 4 days	35	8	400	3 ,	—^{112}
17	22	6	200	3 ,,	—^{112}
12	12	9	250	3 ,,	—^{111}
12	15—31	9	225	3 ,	—^{100}
12 weeks	12—24	9	430	5 DMS	—^{112}
17 ,, 4 days	7	6	180	5 ,,	—^{112}
17 weeks 4 days	7	6	180	5 DiCl	—^{138}
17 4 ,,	7	6	175	5	—^{155}
16 weeks	31	10 approx.	325	2 DMS	—^{110}
24 ,, 3 ,,	C— V+18	10	360	2 ,	—^{105}
—	—	—	169	2	—^{71}D
8 weeks	19		180	3 DMS	—^{57}D
18 weeks 3 days	26	10 approx.	395	5 DMS	—^{112}
18 ,, 3 ,,	21	10	400	5	—^{112}
18 , 3 ,,	29	10	380	5 ,,	—^{112}
18 ,, 3	31	10 ,,	345	5 ,,	—^{112}
18 ,, 3	16	10 ,,	400	5	—^{112}
24 weeks 3 days	C24 V 7	6	300	3 DMS	—^{27}D
24 ,, 3 ,,	C11 V10	7	250	3 ,,	—^{102}
As above		As above			
24 weeks 3 days	C11 V29	6	250	5 DMS	—^{119}
24 ,, 3		7	330	5 ,	—^{140}
24 ,, 3	C31 V10	7			

† D = died

their blood for 7 to 10 weeks after treatment, and throughout this time groups of as many as eight infected flies were fed on each beast every other day or every third day. Anyone acquainted with the difficulties of raising and infecting tsetse flies will appreciate what this means in terms of labour and organization.

TABLE XVI. Experiments on the susceptibility of break-through strains of *T. vivax* (Ensh strain) obtained by repeatedly challenging treated cattle with infected tsetse flies.

Number.	Weight (kg.).	History	Drug treatment prior to break through.	Break through. Days after treatment.	Break through. Days after first challenge.	Treatment of break through mg. per kg. DMS	Result.
6237	255	Clean	—	Sub. from	5026	0.5	R[44]
6240	178		—		4745	0.5	—[48]
2470	215	Also infected with *T. congolense* Muraluma	—		5125	1	R[44]
3506	163		—		5454	1	—
5026	190	Cured *T. congolense* Kenya T 90 challenged *T. congolense* Kenya T 90	0.25 mg. per kg. DrCl	28	78	1	—
5036	200	Cured *T. congolense* Kenya T 90	—	Sub. from	5440	1	—[48]
5040	161	Cured *T. congolense* Kenya T 90 challenged *T. congolense* Muraluma	mg. per kg. DrCl 1 mg. per kg. DMS	66	13	1	—[44]
5107	197			65	12	1	—[44]
5105	180			65	15	1	—[48]
5114	220	Cured *T. congolense* Kenya T 90	1 mg. per kg. DrCl	61	56	1	—[44]
5116	220			61	49	1	R[47]
5117	290			61	49	1	—
5119	245			45	48	1	—[44]
5128	48			47	40	1	—[48]
5440	47			47	40	1	R[48]
5454	200			47	39	1	—
5337	245	Also infected with *T. congolense* Muraluma	—	Sub. from	5117	1	—[48]
4745	450	Cured *T. congolense* Kenya T 90 challenged *T. congolense* Kenya T 90	1 mg. per kg. DrCl	70	67	1.5	—[44]
5129	400	Cured *T. congolense* Kenya T 90		75	68	1.5	—[44]
6247	350	Clean	—	Sub. from	5129	1.5	—[44]
6248	300		—		5129	1.5	—[44]
6244	445		—		4745	1.5	—[48]

The results achieved in treating the break-through strains are given in Table XVI

It will be noted that some of the animals listed in the table were infected by the inoculation of blood taken from those carrying break-through strains The purpose of using subinoculated animals was twofold It was a safeguard against the strain being lost, and it also allowed the influence on the therapeutic result of any tolerance to *T vivax* acquired by the repeatedly challenged bovine to be checked When considering the results in Table XVI, it should be kept in mind that in a curative experiment with the parent strain (Table VI), one of six animals treated with 1 mg per kg DMS relapsed Unfortunately, because of events outside my control, the examination of the treated break-through infections could not be continued as long as was desired, and so a strict interpretation of the results cannot be made If, however, it is conceded that with this Emali strain of *T vivax* relapses after about 2 months (56 days) are infrequent, then it may be said of this experiment that in a group of animals treated with 1 mg per kg DMS three relapsed, and 10 ran negative sufficiently long for their cure to be regarded as likely In any event, it does not appear that these particular break-through strains acquired much resistance to antrycide

(iii) *The susceptibility of break-through strains of* T congolense *obtained from cattle at Kiboko*

For this part of the work it was planned that animals in the field experiment at Kiboko which came to show *T congolense* should be subinoculated into young rats or mice, which should then be sent to my laboratory in Manchester for the susceptibility of the strains to antrycide to be tested It is not possible in such work, of course, to make a comparison between the break-through

TABLE XVII Susceptibility of "clean" and "break-through" strains of *T congolense* to antrycide in mice

Strain	History	Approximate minimum curative dose in mg per kg DMS when tested in mice
S771	"Clean" strain from the Sudan	1
NG3	" Nsongezi	1
NG9	" "	1
K5880	" Kiboko	1
K2554	"Break-through" strain from Kiboko	10
K5831	" " "	8
K5861	" " "	>10
K5866	" " "	10
K5882	" " "	7
K5913	" " "	>10

In none of the cattle which have been treated in these experiments and in none of the laboratory animals (mice, rats, rabbits, monkeys) in which toxicity tests have been done, have delayed deaths been noted.

I have not given horses more than 5 mg per kg antrycide methylsulphate The two that received this treatment tolerated it well but personal communications have been received which state that this same dose has caused much distress in some horses.

Mr A. S. Taylor, of these laboratories working with Mr J T R. Evans in Khartoum, gave big doses of methylsulphate subcutaneously to three camels. One about 5 years old, weighing 227 kg., which received 25 mg per kg was obviously distressed after the injection, and died 34 hours later The second aged 30 or more and weighing 400 kg was given 20 mg per kg and died 6½ hours later The third, an adult male weighing 452 kg was given 15 mg per kg and, although distressed for a short time afterwards quickly recovered. In the course of a curative experiment, eight camels received 10 mg per kg without obvious ill effect.

ADDENDUM I

(1) Camels infected with *T evansi* have been treated with antrycide in laboratory experiments done at Khartoum. A full report of the experiments will be published by Mr J T R. Evans. The main conclusions are as follow

- (a) *Three of five camels treated with 2 mg per kg methylsulphate relapsed two* remained negative for 120 days after treatment.
- (b) Five of five camels treated with 5 mg per kg. methylsulphate remained negative for 120 days after treatment.
- ( ) 5 mg per kg chloride exerts prophylactic effect for at least 2 months, and 10 mg per kg chloride exerts prophylactic effect for at least 4 months. The prophylactic effect of 5 mg per kg methylsulphate is less than months

(2) Two horses infected with *T brucei* were treated with 5 mg per kg chloride one relapsed and one was apparently cured. Two others treated with 5 mg per kg methylsulphate were apparently cured. Several dogs and donkeys also appear to have been cured of *T brucei* with doses of 5 mg per kg methylsulphate or less.

(3) Wilson (1949) has reported that three pigs infected with *T simiae* were cured with respectively 5 mg per kg 4 mg per kg and 3 mg per kg methylsulphate

Conclusions.

1 The curative treatment for three strains of *T congolense* tested in these experiments was a single subcutaneous dose of about 1 mg per kg antrycide *methylsulphate and for a fourth was* about 2 mg per kg The two strains of *T vivax* were not so completely tested, and all that can be said of them is that 1 mg. per kg was not sufficient to cure all animals, but 5 mg per kg was. The latter dose has been suggested *as the field dose because it errs on the generous* side in the treatment of *T congolense* and has cured those strains of *T vivax* which have been tested. A personal communication from Mr J T R. Evans has described how in a big field trial in the Sudan, approximately 200,000 cattle were given this dose with almost entirely satisfactory results, disquiet only arising when calves were being treated.

2 On the basis of the evidence presented here, it is recommended that yearlings and older cattle should not be given more than 12 mg per kg antrycide methylsulphate, and treated animals should be kept as quiet as possible during treatment and for about 12 hours afterwards It should be noted that these big doses (*i e*, 12 mg per kg or more) have been given only to healthy cattle, and it is possible that cattle ill with trypanosomiasis or from some other cause may be less tolerant of the drug

3 The experimental results concerning the treatment of *T brucei* in horses, donkeys and dogs are meagre, and the most that can be said is that 5 mg per kg methylsulphate has been used with apparently satisfactory results in the few cases tried It is a point of importance that while all the treatments have been made as a single subcutaneous dose because this is clearly the treatment of choice for cattle, the necessity for single dose treatment is not so important in horses and dogs

4 All the treatments reported here have been made subcutaneously, but a small number of cattle, and laboratory animals, have been treated intramuscularly It appears that the doses recommended for subcutaneous injection may be safely given intramuscularly should this route be preferred

5 The properties of antrycide chloride are such that they lend themselves to the production of drug-fast strains This salt should not therefore be used alone for curative purposes

6 The prophylactic effect of the methylsulphate is inferior to the chloride, but since it should be assumed that under general field conditions some, at least, of the cattle being treated for prophylactic purposes may be infected with trypanosomes, a mixture of the two salts providing both a curative and a prophylactic action should be used in prophylaxis

7 We must assume that break-through strains, at least of *T congolense*, have acquired some resistance to antrycide, and therefore they must be prevented as far as possible In other words, if cattle are continuously exposed to infection, re-treatment must be made while the majority of animals are still protected from a previous treatment The earliest times after treatment at which trypanosomes have been observed in animals kept in a tsetse area are therefore important In the group of cattle at Kiboko which were treated with gramme 1 5 methylsulphate and gramme 1 chloride *T vivax* was first found in blood smears about 13 weeks after treatment and *T congolense* 21 weeks after treatment, in the group treated with gramme 1 5 methylsulphate and gramme 2 chloride either *T vivax* or *T brucei* was found 13 weeks after treatment, *T vivax* was definitely found 19 weeks after treatment and *T congolense* 20 weeks after treatment.

From what was said in the discussion of the prophylactic experiments, we may presume that break-through trypanosomes are found in blood smears about

a month after infection. Consequently to be fairly assured that re treatment with either of these drug mixtures is made at a time when the great majority of animals are still *completely* free of trypanosomes, it must be made not later than about 8 weeks after the previous treatment. Probably if re-treatment is to be made at so short a time as this then gramme 1·5 methylsulphate mixed with gramme 0·5 chloride would be sufficient.

An alternative is to suppose that *T vivax* may be treated differently from *T congolense* In the first place we have good evidence that the break through strains from cattle which were repeatedly bitten by *G pallidipes* carrying *T vivax* (Emali strain) were not obviously resistant to further treatment with antrycide Secondly we know that many strains of *T vivax* pursue an almost benign course and that even a virulent *T vivax* may be very much reduced in virulence if the course of the infection is checked or impeded in any way

If *T vivax* may be regarded differently from *T congolense* the period between treatments may be increased to about 12 weeks The best treatment to employ in these circumstances is gramme 1·5 methylsulphate mixed with gramme 2 chloride because possibly only one of 11 animals at Kiboko which received this dose was infected at the time a second treatment would be given

8. This consideration of a field dose for prophylactic use has been centred, for the most, around the experiment at Kiboko because our aim is to maintain cattle in a tsetse area and Kiboko may be considered truly representative of a tsetse area but the general conclusions that have been drawn concerning the periods of protection to be expected from the various treatments are borne out by the prophylactic experiments in cattle that were done under laboratory conditions.

REFERENCES

Curd F H S & Davey D G (1949). *Nature* 163 89
——— & ——— (1950). *Brit J Pharmacol Chemother* (in the press)
Lawr, S. G (1931). *Lab. J.*, 7 41
Spinks, A. (1949). *Nature* 163 954
Wilson, S. G (1949). *Vet. Rec* 61 395

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ON THE DISSECTION OF MOSQUITOES FOR MALARIA PARASITES AND THE INFORMATION TO BE DERIVED THEREFROM *

BY

ERNEST P HODGKIN †

Department of Zoology, University of Western Australia.

The dissection and examination of wild caught mosquitoes for the presence of malaria parasites is standard practice among malariologists The results are used to establish particular species of anopheles as vectors of malaria and, when expressed as sporozoite rates and oocyst rates, they are regarded as indicative of the intensity of transmission that is taking place

The figures that have been published in the literature on the transmission of malaria vary greatly Differences in the bionomics of the vector mosquitoes and in the human reservoir of infection will affect the proportion of the mosquitoes that is found infected, but there is more variation than experience would lead one to expect It is the thesis of this paper that published rates cannot be compared one with another because of differences in the technique of examining mosquitoes , in particular, the practice of keeping freshly engorged mosquitoes for several days before they are dissected invalidates many infections that are recorded, and therefore also the rates that are calculated from them These points are illustrated by examples from my own investigations made in Malaya between 1931 and 1941

The paper which follows was written in 1941 and was almost complete when the Japanese captured Singapore No copies appeared to have survived until, by a fortunate chance, one was found recently in Malacca I see no reason for altering my views as expressed in 1941, and although the paper has been redrafted no essential alterations have been made to it

* I wish to acknowledge gratefully the helpful criticism I have received from my colleagues, first at the Institute for Medical Research and then in the Zoology Department of the University of Western Australia, also the loyal co-operation of my staff in the investigations used here as examples

† Formerly Entomologist at the Institute for Medical Research, F M S

a month after infection. Consequently to be fairly assured that re-treatment with either of these drug mixtures is made at a time when the great majority of animals are still *completely* free of trypanosomes, it must be made not later than about 8 weeks after the previous treatment. Probably if re-treatment is to be made at so short a time as this then gramme 1·5 methylsulphate mixed with gramme 0·5 chloride would be sufficient.

An alternative is to suppose that *T. vivax* may be treated differently from *T. congolense*. In the first place we have good evidence that the break-through strains from cattle which were repeatedly bitten by *G. pallidipes* carrying *T. vivax* (Emali strain) were not obviously resistant to further treatment with antrycide. Secondly, we know that many strains of *T. vivax* pursue an almost benign course and that even a virulent *T. vivax* may be very much reduced in virulence if the course of the infection is checked or impeded in any way.

If *T. vivax* may be regarded differently from *T. congolense* the period between treatments may be increased to about 12 weeks. The best treatment to employ in these circumstances is gramme 1·5 methylsulphate mixed with gramme 2 chloride because possibly only one of 11 animals at Kiboko which received this dose was infected at the time a second treatment would be given.

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REFERENCES

Curd, F. H. S. & Davey, D. G. (1949). *Nature* 163, 89.
——— & ——— (1950). *Brit. J. Pharmacol. Chemother.* (in the press).
Laws, S. G. (1951). *Lab. J.* 7, 41.
Spinks, A. (1949). *Nature* 163, 954.
Wilson, S. G. (1949). *Vet. Rec.* 61, 395.

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THE PURPOSE OF DISSECTING MOSQUITOES.

The primary object of examining wild caught mosquitoes for malaria parasites is to establish the identity of the anopheles vectors (species transmitting malaria in the field) and to evaluate the relative importance of the different species as vectors.

1 The presence of parasites in an infective stage *i.e.* of sporozoites in the glands, will incriminate species of anopheles as vector When the results of dissections, based on suitable field samples, are consistently negative it may be assumed that the species is innocuous. Oocysts are not infective to man, and their presence in mosquito not proof that it is transmitting malaria.

2 The sporozoite rate is measure of the infectiveness of the vector or vectors in particular environment. (The sporozoite rate is the percentage of female anopheles with sporozoites in the salivary glands.)

3 The oocyst rate is indicative of the infectiveness of the human community to mosquitoes. (The oocyst rate is the percentage of female anopheles with oocysts in the gut wall.)

If the figures for gut and salivary gland infections are totalled and gross infection rate calculated, the resultant figure obscures the significant information derived from the separate rates.

4 An estimate of the intensity of transmission in locality may be made from the sporozoite rate if the number of vector mosquitoes that will bite person in given time is known.

EXAMPLES OF THE DISSECTION OF MOSQUITOES.

These examples from Malaya illustrate the following specific points which will be discussed in a later section. (*a*) The falsification of infections and infection rates when mosquitoes are kept for some days between capture and dissection (Examples 1 to 5) (*b*) the misinterpretation of data with regard to the biology of the mosquitoes resulting from the same cause (Examples 3, 6 and 7) (*c*) the effect on the results of the method of capture (Example 8) (*d*) Examples 9 to 13 are illustrative of information relative to the intensity of transmission of malaria, and similar information is also drawn from Examples 1 and 2.

In the examples cited below it may be assumed that the great majority of the mosquitoes described as dissected "0 to 3 days after capture" were caught without blood in the gut, while of those dissected "4 or more days after capture," most were caught following a recent blood meal and were kept for 7 to 9 days before dissection.

Example 1 HODGKIN and JOHNSTON (1935) report the finding of naturally infected *Anopheles barbirostris* v d. Wulp at Batu Gajah, Perak during 1933 and 1934 The town of Batu Gajah lies between low hills on one side and swamps on the other Extensive anti-malarial measures prevented the breeding of *A maculatus* but the swamp-breeding anopheles went uncontrolled. There was little malaria by rural standards, 182 cases were reported in 1934 in a population of 6 759 (1931 census). During 1933 catches were made by hand in human habitations and of the 839 *A. barbirostris* dissected and examined for malaria parasites 2 4 per cent. were found with oocysts, and 1 2 per cent. with sporozoites in the

glands In the following year a human bait trap* was used, and in the 691 *A barbirostris* dissected the oocyst rate was only 0 6 per cent and the sporozoite rate 0 6 per cent

Two conclusions might be drawn from the above results as they stand, first, that there was less transmission during 1934 than in 1933, and second, that for some reason mosquitoes caught in a trap show a lower percentage of infection From the more detailed analysis of the figures given in Table I it is clear that neither of these conclusions is justified

TABLE I *Anopheles barbirostris* from Batu Gajah Perak

Dissected, days after capture	Total dissected	Oocysts present		Sporozoites in glands	
		Total	Per cent	Total	Per cent
1933 hand catches					
0 to 3 days	412	3	0.7	0	0
4 or more days	427	17	4.0	10	2.3
Total	839	20	2.4	10	1.2
1934 trap catches					
0 to 3 days	637	1	+	3	0.6
4 or more days	54	3	+	1	–
Total	691	4	0.6	4	0.6

About half of the mosquitoes that were caught by hand in 1933 were freshly engorged and were kept in the laboratory for a week or more before dissection, most of the gut and all of the gland infections were found in these mosquitoes When, in 1934, a trap was used very few of the mosquitoes so caught contained blood and for this reason nine-tenths of the mosquitoes were dissected within 3 days of capture, of these three showed sporozoites in the glands

In Table VII only the 1934 figures are given (*i e*, mosquitoes taken in the trap), and only the mosquitoes dissected within 3 days of capture are included

Example 2 Mosquitoes were trapped nightly for nearly 3 years (1931–1934) on a rubber plantation where *A maculatus* Theo was the vector of malaria, with the purpose, among other things, of estimating the intensity of transmission There were no anti-malarial measures worth the name There was severe endemic malaria among the Indian labour force and dependants

* The human bait trap consists of a large mosquito net surrounding a camp bed screened with a smaller net The operator rolls up a door in the large net and lies on the bed, at specified intervals he gets up, closes the door, and catches all the mosquitoes inside the large net The catches are usually made either at 8 and 10 p m, midnight, 2, 4 and 6 a m, or at 7, 8, 9, 10 and 11 p m (For a full description, see GATER, 1935, or HODGKIN, 1946)

(December 1931 spleen rate 43 per cent. parasite rate 32 per cent. persons examined 180) The dissection of the *A. maculatus* which were caught produced the results shown in Table II It will be seen that there are three different sporozoite rates on which to base an estimate of the intensity of transmission the gross rate, 0·7 per cent. the rate in mosquitoes dissected within 3 days of capture 0 5 per cent. and the sporozoite rate in mosquitoes kept in the laboratory for 4 or more days, 1 1 per cent.

TABLE II *Anopheles maculatus* from Estate X, Selangor

	When dissected.		Total dissected
	0 to 3 days after capture.	4 or more days after capture	
Total dissected	7,315	2,570	9,885
Number with oocysts	12	20	32
Number with sporozoites	37	28	65
Total infected	48	42	90
Oocyst rate per cent.	0 2	0 9	0·3
Sporozoite rate per cent.	0 5	1 1	0 7

The 1932 figures alone have been included in Table VII and they have been broken up so as to separate the catches during the period March and April. All mosquitoes kept in the laboratory for more than 3 days have been disregarded. (Ann. Rep., I M R. 1931–34.)

Example 3 The figures in Table II refer only to mosquitoes that were caught in a human bait trap A further 600 *A maculatus* were caught in an automatic trap which the mosquitoes entered on leaving the labourer's rooms, and these were also dissected with the following gross results

Total dissected	600	Total infected	16
Number with oocysts	6	Oocyst rate per cent.	1·0
" sporozoites	10	Sporozoite rate per cent.	1 7

The gross sporozoite rate here is 1 7 per cent. compared with 0·7 per cent. in the *A. maculatus* taken in the human bait trap Of those caught in the automatic trap 76 per cent. contained blood in the stomach when captured, and were kept for 4 or more days before dissection, while only 26 per cent. of those caught in the human bait trap had recently fed.

Example 4. From the same estate a considerable number of *A. karwari* James was dissected (3,734). Four of them were found to be infected two with oocysts and two with sporozoites in the glands. All these four mosquitoes had been kept in the laboratory for from 7 to 9 days before dissection.

Example 5 One hundred *A. maculatus* were caught on a plantation where it was believed that certain persons had contracted malaria they were caught

by hand, some at night and some in the early morning, and they were kept for periods of a week or more to allow the blood they contained to be digested When they were dissected, 28 out of the 95 examined were found to be infected with malaria parasites, seven of them in the salivary glands, a sporozoite rate of 7 per cent. (Ann Rep, I M R, 1931)

Example 6 The ovaries of the mosquitoes trapped at Batu Gajah (Example 1) were examined and classified on the scale described by CHRISTOPHERS (1911) *

Table III shows the percentages of three species of anopheles which were found to have the ovaries developed beyond Stage II of CHRISTOPHERS, that is to say, the ova were in process of maturation following a blood meal The gross figures in the last column show a much greater percentage with maturing ova (Stages III to V) in 1933 than in 1934 However, it is evident from the preceding columns that the proportion of mosquitoes kept for more than 3 days, because of a recent blood meal, was much larger in 1933 than in 1934

TABLE III Anopheles from Batu Gajah, Perak
Showing numbers dissected and percentage found with ova developed beyond stage II of CHRISTOPHERS

	Dissected, days after capture					
	Number dissected.			Per cent. with maturing ova		
	0 to 3 days	4 or more days	Total	0 to 3 days	4 or more days	Total
A aconitus						
1933	304	175	479	8	65	29
1934	411	41	452	2	27	4
A barbirostris						
1933	412	427	839	24	89	58
1934	637	54	691	6	44	9
A hyrcanus vars						
1933	202	147	349	9	63	32
1934	817	63	880	2	29	4

(From Table III it appears that there was also a real difference between the figures from the 2 years but it would be necessary to analyse the figures more thoroughly before drawing conclusions)

* This is an artificial scale to describe the development of the ovarian follicle Stage I is found only in nulliparous mosquitoes, the ova are without yolk Stage II ovari may be found in nulliparous females or those that have laid eggs but have not yet beg to develop a second batch In Stages III and IV the ova are in process of maturatio and in Stage V the ova are mature

Example 7 During 1939 nearly 2,000 *A. letifer* Gater were caught in a human bait trap on the Selangor coast 1 139 were caught without blood and were dissected within 3 days of capture and only 5 per cent. of them were found with maturing ova, while a further 351 were caught with blood in the gut and were dissected 4 or more days after capture of these all but two had maturing ova.

Example 8. At one locality on the Selangor coast *A. baezai* Gater is easily caught during the daytime resting at the base of "Nipah" palm fronds. During 1940 weekly catches were made in this way and a human bait trap was operated regularly in a nearby hut. (Ann. Rep. I.M.R., 1940.) The mosquitoes were dissected within 48 hours of capture, usually less, with the results shown in Table IV

TABLE IV *Anopheles baezai* from trap and daytime resting places.

	Number dissected.	Oocyst rate per cent.	Sporozoite rate. per cent.
Caught on Nipah.	2,280	3·4	3·8
Human bait trap	783	1·6	1·6

TABLE V Anopheles trapped at Kampong Jeram.

	Number caught.	Number dissected.	Oocysts present, total.	Sporozoites in glands, total.
A. barbirostris	3 120	2 848	9	9
A. letifer	530	480	—	1
A. sundaicus	689	640	2	—
Other anopheles	314	263	—	—

Example 9 Mosquitoes were caught in a human bait trap at Kampong Jeram, a Malay settlement on the Selangor coast, between February 1935 and February 1937 *A. barbirostris* was the principal species caught but smaller numbers of *A. letifer* and *A. sundaicus* were also taken, as well as non-vector species. As indicated by the child spleen rate, the area was one of high malarial endemicity The gross results of the trapping and dissection are shown in Table V (the discrepancy between these figures and those shown in Table VII is due to the fact that the figures for January and February 1937 were no longer available when T ble V was prepared).

There is an error due to the inclusion of mosquitoes kept for more than 3 days which does not exceed 25 per cent. of the calculated figures in Table VII

Example 10. Mosquitoes were trapped at Kampong Sijangkang, a Malay

settlement on the Selangor coast, between August 1936 and April 1940 *A letifer* was almost the only species of anopheles caught The trap was operated at several sites, the catches being much larger at " C " than at the other sites To judge from the parasite rate (44 per cent.) and spleen rate (71 per cent), malaria was highly endemic in the settlement as a whole In Table VII the figures given for sites A and B may be 25 per cent too high, but for site C they are accurate (Ann Rep , I M R., 1936–40)

Example 11 Mosquitoes were caught between March 1937 and October 1940 on a tea estate on the Selangor coast where there were no anti-larval measures The estate is on an isolated patch of hill land entirely surrounded by jungle swamp in which *A umbrosus* Theo bred unrestricted, while *A letifer* bred freely in the drains round the estate boundary The Indian and Javanese labour force and dependants received prophylactic plasmoquine or atebrin for periods of a year, and 15 months respectively , the intervening periods when there was no prophylaxis totalled about 20 months Malaria was hyperendemic before prophylaxis began, but the incidence was greatly reduced by prophylaxis and by treatment in the control group (Ann Rep , I M R , 1936–40)

The results of the trapping and dissection are shown in Table VII Some mosquitoes kept for more than 3 days are included and may cause an error of up to 25 per cent in the case of the " *A umbrosus* no prophylaxis " and " *A letifer* prophylaxis " The other figures are unaffected

Example 12 Mosquitoes were caught on a coconut estate in Lower Perak on the south bank of the Perak river, near the upstream limit of brackish water The catches were made between November 1939 and October 1941 The Indian labour force was housed close to the river bank and the breeding of anopheles larvae was controlled by oiling within a semi-circle of over half a mile from their quarters , outside the oiled area there was intense breeding of *A sundaicus* Rdnw Other species were caught in negligible numbers There was comparatively little clinical malaria, but the arrival of a small number of non-immune labourers in June 1940 was followed by an increase in the incidence of malaria Spleen and parasite rates in children between the ages of 2 and 12 years were as follows

Date	Number examined.	Parasite rate per cent.	Spleen rate per cent.
October, 1939	122	40	32
July, 1940	114	69	43
September, 1940	136	55	38

The figures in Table VII are not affected by mosquitoes having been kept in the laboratory for more than 3 days

Example 13 Mosquitoes were caught at Kampong Rantau Panjang, a

Malay settlement on the Selangor coast, between February 1935 and December 1937. As indicated by the child spleen rate, the malaria was of moderate endemicity. The common species of anopheles were *barbirostris*, *hyrcanus*, *kochi*, *sundaicus*, and *vagus*. Oocysts were found in both *A. sundaicus* and *A. barbirostris*: there were no sporozoites and the identity of the vector remains in doubt. Three thousand *A. sundaicus* were dissected but, with a sporozoite rate of only 0·04 per cent., it would have been quite possible to dissect this number and find none with sporozoites.

Example 14. Hypothetical case. The point that has been made in the earlier examples with regard to the effect of keeping mosquitoes in the laboratory for some days before dissection will be made clearer by the examination of a purely hypothetical history of a batch of wild caught anopheles. This is illustrated in the Figure. For the purpose of this example, the following assumptions have been made:

(a) All mosquitoes fed on persons carrying infective gametocytes. (b) of the mosquitoes caught, half had emerged as adults and had their first blood meal on the night of capture, while the remainder had emerged and fed at intervals of 4 days, as indicated on the left of the diagram; (c) the parasites developed at a uniform speed, sporozoites appearing in the glands of all the mosquitoes by the 12th day after the infecting feed.

The results that would be obtained by dissecting the mosquitoes 4, 8, and 12 days later are shown on the right of the diagram. The mosquitoes that had fed more than once would be found with parasites in various stages of development but, for simplicity, only the oldest parasites are indicated in the figure. The results of the dissections are summarized in Table VI.

TABLE VI. Hypothetical Example. Results of dissecting mosquitoes.

When dissected.	Oocyst rate per cent.	Sporozoite rate per cent.
Immediately after capture	40	1[illegible]5
4 days after capture	100	25
8	100	50
12	0	100

The dissections made 4, 8, and 12 days after capture have thus given fictitious oocyst and sporozoite rates, if the purpose is to assess the infectiveness of the mosquitoes to man. The effect of keeping the mosquitoes in the laboratory has been to increase the sporozoite rate from 12 per cent. at the time of capture to 100 per cent. when they have been kept for 12 days.

DISCUSSION.

Two basic assumptions are usually made with regard to the malaria parasites found in wild caught mosquitoes. First it is assumed that the presence of apparently normal sporozoites in the salivary glands is evidence that the

mosquito is in an infective condition From experience with experimental infections it would appear that the assumption is substantially true, but it must be appreciated that such experience provides no more than a tentative working hypothesis Secondly, it is assumed that the parasites found belong to a species of *Plasmodium* that will infect man The assumption must, however, be examined in reference to each locality where an investigation is made In

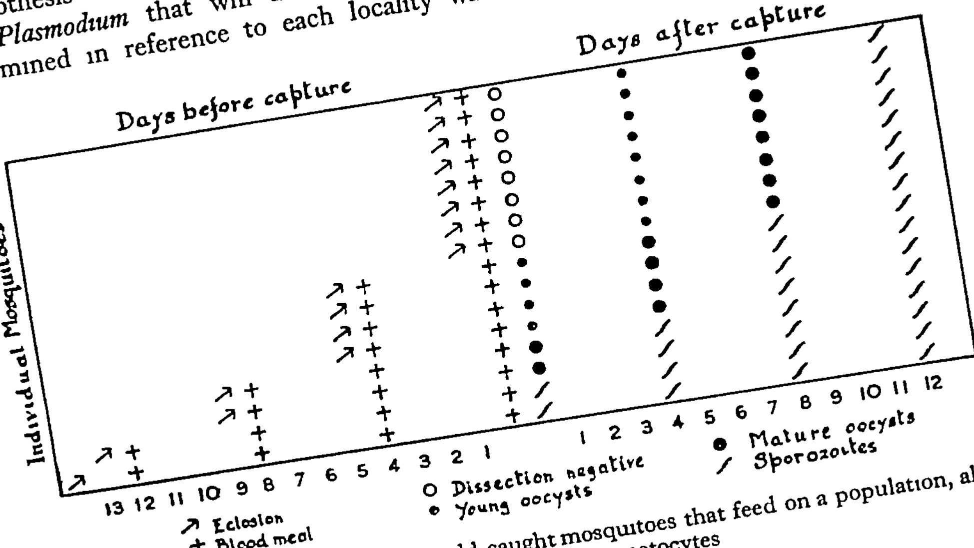

FIGURE —The history of a batch of wild caught mosquitoes that feed on a population, all of whom carry infective gametocytes

my Example 8, doubt arose about the identity of the parasites that were seen, and for this reason *A baezai* is only tentatively regarded as a vector In Example 11 it is possible that the parasites found in the jungle-haunting *A umbrosus* belonged to species of monkey malaria It is probable that in the other examples the parasites were of human origin

The Effect of Keeping Mosquitoes in the Laboratory before Dissection

Anopheles mosquitoes are frequently kept in the laboratory for many days before they are dissected , in reports this is sometimes explicitly stated, but more often it can only be inferred from the context In fact, the advice is often given to keep mosquitoes caught with blood a long enough period for the blood to be digested and for immature parasites to develop into recognizable oocysts The effect of this in producing artificial infections has been illustrated in the foregoing Examples

1 In the simplest case the result of holding the mosquitoes in th laboratory can be to incriminate a species as a vector on false evidence Example is of this nature *A karwari* is widespread and abundant in Malaya, being mo

or less co-incident in distribution with the important malaria carrier *A. maculatus*. There is no epidemiological evidence for suspecting that *A. karwari* is a vector of malaria and this is the only published record of infections having been found in wild caught mosquitoes. The four infections, two gut and two gland, were all found in mosquitoes that had been kept in the laboratory for a week or more not one mosquito that was dissected within 3 days of capture (the bulk of the dissections) was found with malaria parasites. It must be concluded that there is no reason to believe there were sporozoites in the glands of any of these mosquitoes at the time of capture and therefore there is no evidence that *A. karwari* is a vector of malaria.

The same statement might be made with regard to *A. barbirostris* on the evidence of the 1933 figures given in Table I of Example 1 Three out of the 412 mosquitoes dissected within 3 days of capture showed oocysts on the gut, but none carried sporozoites All the gland infections were found in mosquitoes kept for 4 or more days On the other hand, the finding of the three gland infections in 1934 is positive evidence that *A. barbirostris* is a vector the man operating the trap would, had he not been protected by a mosquito net, have been exposed to the bites of at least three infective *A. barbirostris* during the course of his work. It is relevant to note that subsequent investigations have shown the existence of two forms of *A. barbirostris* in Malaya, and that of these one is a vector while the other is probably harmless (REID, 1941). At Batu Gajah a mixture of the two forms was caught.

It may seldom happen that a species will come to be considered a vector on such evidence but the consequences of wrong identification can be serious. It not infrequently happens that the real vector in a locality is either relatively rare or wild in its habits and so it may be discounted while other species which are abundant may come to be regarded as the true vectors because they feed on man sufficiently frequently for infections to be found in them when they are kept for several days before dissection For many years *A. maculatus* was believed to be the principal vector in Borneo because it was common and was known to transmit malaria in the Malay peninsula. Recently MCARTHUR (1947) has shown that it is probably harmless and that the real vector is *A. leucosphyrus* Dön. which is a shy species that is rarely caught by orthodox methods.

2. If the sporozoite rate is to be used as a measure of the infectiveness of the vector or vectors to the human population, it must be based upon a sample of the mosquitoes that are actually biting man. For this reason, in the hypothetical Example (14) the mosquitoes dissected 4, 8 and 12 days after capture give no information about the risk of infection to which a person was exposed on the night the mosquitoes were caught.

The argument has been put to me that if these mosquitoes had not been caught the parasites they acquired at the infecting meal would have developed and the mosquitoes would have become infected in the same way that they

have done in captivity This is not true An unknown proportion would have survived to do so and in the meantime the sample would have been diluted by an influx of freshly emerged adults Under stable conditions the composition of the total population will remain constant from night to night and, within the limits of experimental error, successive samples of freshly caught mosquitoes will give the same results on dissection The age distribution of a human population is analagous, this remains constant from year to year, within limits comparable with those of the mosquito population under consideration The proportion of the mosquitoes that will survive long enough for sporozoites to mature is one of the essential unknowns in the problem of malaria transmission, it may be expected to vary with the species, with the season, and with other factors

In the actual examples cited above the differences between the sporozoite rates of the mosquitoes dissected within 3 days of capture and those dissected 4 or more days later is marked Individually the differences are not, as presented, statistically significant but their repetition leaves no doubt that many of the gland infections would have been recorded as gut infections or not at all if the mosquitoes had been dissected immediately In Example 2, with nearly 10,000 *A maculatus*, the effect of keeping the mosquitoes has been to double the sporozoite rate (0 5 per cent and 1 1 per cent) In Example 1, a fictitious sporozoite rate of 1 2 per cent was recorded in 1933, and there is no means of knowing what was the true rate In Example 5 the fantastic sporozoite rate (by Malayan standards) of 7 per cent was found which, in the circumstances, tells us only that the human population was highly infective to mosquitoes

Thus the effect of keeping the mosquitoes in the laboratory is to render invalid the calculated sporozoite rate, the measure of the infectiveness of the vectors to man

3 From Example 6 it will be seen how a similar false conclusion may be drawn with regard to the biology of the mosquitoes From a comparison of the hand catches (1933) with the trap catches (1934), it might have been concluded that there is a difference in regard to the maturation of the ova between mosquitoes found in houses and those that enter the trap, that in the former the ova mature readily, while in the latter they do so only in a much smaller proportion The error is here so crude that it could hardly have been overlooked, but if the figures had been more nearly alike it might have passed unrecognized

Example 7 serves only to re-emphasize the same point Here there is no possibility of false conclusions being drawn because there was no separation in time or place between the mosquitoes dissected immediately and those retained in the laboratory

In Example 3 the higher percentage of infections in the *A. maculatus* caught with the automatic trap as compared with the human bait trap might have been taken as evidence either that mosquitoes stay in houses during the day while actually they rarely do so, or that they have a homing instinct. In fact, the difference between the two infection rates is to be attributed to the different proportions of the two sets of mosquitoes that were kept in the laboratory before dissection.

4 If the oocyst rate is used as a measure of the infectiveness of the human population to mosquitoes, it would seem to be logical to retain the mosquitoes in the laboratory long enough for the parasites to become recognizable. However dissections are rarely made with this purpose in view since the gametocyte rate supplies an estimate that is generally easier to obtain and more informative

Other Factors that may affect Dissection Results

The foregoing makes it clear that statistics based on the dissection of specimens kept in the laboratory for several days are a major source of error but there are other matters to which attention should be drawn when the results of dissections are reported. These relate to the time place and method of capture of the mosquitoes.

In Malaya it is generally found most satisfactory to catch anopheles by means of the human bait trap because they are difficult to find in houses during the day sometimes it is possible to make hand catches in houses at night or in the early morning and for certain purposes it is useful to catch mosquitoes in cattle sheds elsewhere hand catching by day in houses is commonly resorted to. It is not to be expected that the different methods of sampling will give the same results and it is important in reports to make clear how the sample was obtained.

In Example 8 above, the contrast between the oocyst and sporozoite rates of the *A. barbirostris* caught in the trap with those taken on the palm fronds is marked. To have lumped the figures would have been to give false rates and to obscure an interesting and important difference It is reasonable to assume that the sample of mosquitoes caught in the trap attracted to a human being, more closely resembles the population that is attacking man than does the sample caught from the daytime resting places

The necessity for recording the time and place of capture are more obvious. Both the time of year and the time of day at which catches are made may affect the results. In figures for *A. maculatus* given below (Table VII) it will be seen that the sporozoite rate during the transmission season was double that during the rest of the year With regard to place it is clearly desirable to give information on the human population, and particularly the extent to which it is infected with malaria, though for practical reasons it is not always possible to do this.

THE USE OF THE SPOROZOITE RATE IN ESTIMATING THE INTENSITY OF TRANSMISSION

The sporozoite rate alone is of little value in assessing the part played by a particular species in transmission or estimating the malaria risk to which man is exposed, even when the data are collected with the precautions here prescribed It is necessary to know also how many of the vector species will bite an individual in a given period This is emphasized by DAVEY and GORDON (1933), who draw attention to the importance of estimating the density of infective anopheles as a means of calculating the risk of inoculation with malaria Whether their methods of estimating the "infective density" of anopheles are used or not is immaterial, but if full value is to be obtained from dissection results some estimate must be made of the frequency with which man is liable to be reinfected with malaria For this purpose it is desirable to know, in addition to the sporozoite rate, approximately how many of the vector species of anopheles will bite man each night during any given period Because of the absence of this information the "infective density" of DAVEY and GORDON is, as stated by them, a comparative estimate only

As already mentioned, the human bait trap has been found to be the most satisfactory method of capturing anopheles in Malaya It is believed that the results obtained with this type of trap give an approximation of the number of anopheles mosquitoes that will attack an unprotected person The data obtained with this trap may therefore be used to make a direct estimate of the intensity of transmission If the sporozoite rate (a percentage) found in a particular species is multiplied by the average nightly catch of that species the resultant figure will be a measure of the number of infective bites which an exposed individual may expect to receive from that species in a hundred nights in the locality

In the Examples (1, 2, and 9 to 13) I have given information of this nature relative to the five principal Malayan vectors of malaria In every case the mosquitoes were caught by means of the human bait trap

The results are summarized in Table VII, which gives a comparison between the intensity of transmission with these vector anopheles under the particular conditions of the investigations Unfortunately, most of the catches were made before the importance of early dissection was appreciated, in some cases it has been possible to eliminate this source of error, and where this could not be done I have indicated the maximum error under the respective examples described above In no instance is it great enough to invalidate the necessarily very approximate estimates of intensity of transmission that have been given It should be stated that in every case both the numbers caught and the gland infections found were fairly evenly distributed over the periods of the investigations, had this not been so the estimates would be of little interest (*A sundaicu*... is a possible exception) For this reason also no estimate of the statistical sign... ficance of the available figures can be made and none has been attempted

A. barbirostris REID (1941) has shown that there are two forms of this species, and that while his "dark winged" form is a vector the "light winged" form does not appear to transmit malaria. Both forms were taken at Batu Gajah and at Kampong Jeram. For this reason the true sporozoite rate may well have been higher than the figure given. The transmission rate at Batu Gajah appears excessive when compared with the figures for malaria, but the latter refer to the town as a whole while the trap was sited close to the swamps which were the source of the *A. barbirostris*.

A. maculatus The March-April period has been separated because this was the period of greatest transmission, immediately preceding the main malaria season.

TABLE VII. Examples of malaria transmission by Malayan anophelines.

Example number		Total caught	Average per night.	Number dissected.	Sporozoite rate per cent.	Estimated number of infective bites per year
	A. barbirostris					
1	Batu Gajah, 1934	533		437	0.5	15
9	Kampong Jeram	3,236	11	2,890	0.3	12
2	*A. maculatus*					
	Jan.–Mar. 1932	947	12	721	0.6	4
	Mar.–Apr.	1,005	34	738	1.1	150
	May–Dec.	2,637	11	2,002	0.5	70
10	*A. letifer* Syangkang					
	Sites A and B	544	3	718	0.4	4
	Site C	310	1	1,902	0.8	34
11	Tea Estate					
	No prophylaxis					
	A. letifer	2,441	5	2,24[illegible]		15
	A. umbrosus	1,604	2	1,493		5
	Both species	4,335	5	3,741		20
	During prophylaxis					
	A. letifer	3,851	6	3,203	2	2
	A. umbrosus	1,452	2	1,335	2	1
	Both species	5,303	8	4,630	—	4
	A. sundaicus					
12	Lower Perak	23,934	89	10,935	1	12
13	Rantau Panjang	2,05[illegible]		2,97[illegible]	—	—

A letifer The reason for the difference between the two transmission intensities at Sijangkang must be attributed mainly to the greater numbers caught The numbers caught at sites A and B were too small for the lower sporozoite rate to have any meaning

The results of the trapping on the tea estate illustrate two interesting points Firstly, there is a contrast between the intensity of transmission during periods when the population was receiving prophylactic drugs and when there was no prophylaxis The nightly average of vector mosquitoes remained the same but the sporozoite rate was lower during the period of prophylaxis than when there was none Secondly, despite the fact that the sporozoite rates in the two species do not differ significantly it is evident that *A letifer* was playing a much greater part in transmission than was *A umbrosus* An important practical conclusion follows, namely, that control of *A letifer* alone would have eliminated three-quarters of the transmission of malaria on the estate (control of this species would be practicable, while control of *A umbrosus* would be difficult and very costly) It should be pointed out that if these mosquitoes had been kept in the laboratory for some days no valid comparison could have been made

The possibility has been mentioned above that some of the parasites found in the *A umbrosus* were not of human origin but belonged to species infecting monkeys, and this must be taken into account in assessing the value of the sporozoite rates There is, however, little doubt that this species is a vector of malaria (Reid and Hodgkin)

In the foregoing examples the sporozoite rate lies between 1 1 and 0 4 per cent (disregarding the mixed *A barbirostris* catches and the figures affected by prophylaxis) In Example 12, on the other hand, the sporozoite rate for *A sundaicus* was found to be only 0 04 per cent All infections were found in the 10 months following the arrival of the non-immune labourers It is possible that with a stable population the sporozoite rate might have been even lower and, on the other hand, it might have been higher with continual importation of non-immunes This fact reduces the value of the sporozoite rate and the index of transmission intensity Despite this it is useful to state that in this example the intensity of transmission with *A sundaicus* is of the same order as was found with the more heavily infected species because the much greater numbers of *A sundaicus* which occurred amply compensate for the much lower sporozoite rate in that species

From the point of view of the control of malaria, it is significant that these large catches were made in spite of efficient anti-larval measures carried out within half a mile of the trap

CONCLUSIONS.

The examples I have given demonstrate that maximum value can be obtained from the dissection of wild caught mosquitoes only if those that are actually biting man are dissected.

In particular the practice of keeping mosquitoes in the laboratory for some days before dissection produces artificial infections, and the results may be no more informative than those obtained by artificial feeding on gametocyte carriers. They can, in fact, be misleading a common species may be identified as the vector when, in fact, it is harmless, while the true vector which is less abundant or more difficult to catch, may pass unrecognized.

For this reason I suggest that it should be made standard practice to dissect *all wild caught mosquitoes as soon as possible after capture whether they are* recently engorged or not, and that in normal practice they should never be kept for more than 72 hours before dissection.

In publishing the results it is also necessary to give information relative to the time, place, and method of capture of the mosquitoes because all of these may affect the sporozoite rate.

Today the principal vectors of malaria are well known. What is now required is an accurate assessment of the part played by each in transmission relative to the incidence of malaria in the human population. The intensity of transmission by a vector in a locality is a factor of both the percentage that is carrying sporozoites in the glands and the numbers that are actually biting man. In the literature on the transmission of malaria sporozoite rates are often published without any indication of how they were obtained and rarely is there any estimate of the numbers of the vector mosquitoes that are biting man.

The most direct method of obtaining such an estimate is to catch the mosquitoes as they bite. This has certain obvious disadvantages which generally make it undesirable, not least of these is the risk *of contracting malaria to which* the catcher is exposed. In Malaya the human bait trap has been found to provide a convenient means of obtaining the estimate elsewhere other methods may be *found more suitable.*

The estimated number of infective bites received by a person in a year provides a simple index of the transmission of malaria. It may be calculated directly if both the sporozoite rate and the number of mosquitoes biting man are known. This index, together with data as to the seasonal distribution of the infective mosquitoes, can then be studied in conjunction with data on malaria in the human population to give fundamental information relative to the epidemiology of malaria.

SUMMARY

(1) The purpose of dissecting wild caught anopheles for malaria parasites is discussed relative to the incrimination of vector species, the sporozoite rate, the oocyst rate, and an estimate of the intensity of transmission

(2) A series of examples from Malaya and a hypothetical example are given to illustrate certain specific points

(3) The practice of keeping mosquitoes for some days between capture and dissection is shown to produce artificial infections This may result in a species being identified as a vector of malaria when, in fact, it is harmless It will also produce fictitious sporozoite rates which will invalidate any estimate of the intensity of transmission. Further, any information that is extracted with regard to the biology of the vectors (development of ovaries, feeding habits, etc) is liable to be invalidated

(4) For these reasons it is essential to adopt the rule that all mosquitoes, whether recently engorged or not, be dissected as soon as possible after capture A maximum of 72 hours is suggested For studies on the biology of the insects themselves, even this is too long

(5) When both the true sporozoite rate and the numbers of the vector species that are attacking man are known, a useful index of the intensity of transmission can be obtained by multiplying the sporozoite rate by the nightly average catch

(6) In the series of examples given, the sporozoite rate ranged only between 1 1 per cent and 0 4 per cent in the anopheles species *barbirostris*, *letifer*, *maculatus*, and *umbrosus* In *A sundaicus* the sporozoite rate was much lower The index of transmission varied greatly because of the very different numbers of the vector mosquitoes that were caught Very large catches of *A sundaicus* resulted in a rate of transmission of the same order as that found in the other species

(7) The examples are illustrative of the type of information that is made available by the dissection of wild caught anopheles when proper care is taken in their collection and an estimate is made of the numbers that are likely to bite man Examined in conjunction with accurate information on malaria in the human population, the index of transmission will give valuable information relative to the biology of malaria

(8) Other factors that may affect the conclusions drawn from dissection data are considered briefly These relate to the time, place, and method of

capture of the mosquitoes. The importance is stressed of recording all relevant information in reports if full value is to be obtained from them.

REFERENCES

CHRISTOPHERS, S. R. (1911). Reproduced in COVELL, G. (1941). *How to Do a Malaria Survey. Health Bulletin*, No. 14. Delhi.

DAVEY, T. H. & GORDON, R. M. (1933). *Ann. trop. Med. Parasit.*, 27, 27.

GATER, B. A. R. (1935). *Aids to the Identification of Anopheline Imagines in Malaya.* Singapore. Government Printing Office.

GREEN, R. & GATER, B. A. R. (1931). *Bull. Inst. Med. Res., F.M.S.* No. 4 of 1931.

HODGKIN, E. P. (1934–41). In *Ann. Repts. Inst. Med. Res. F.M.S.* 1931 to 1940.

—— (1939). *The Anopheles of Malaya*, Perth, Western Australia.

—— & JOHNSTON, R. S. (1935). *Bull. Inst. Med. Res., F.M.S.* No. 1 of 1935.

MCARTHUR, J. (1937). *Trans. R. Soc. trop. Med. Hyg.* 40, 537.

REID, J. A. (1931). *J. Malayan Branch B.M.A.*, 5, 71. (Reprinted (1937) in *Med. J. Malaya*, 2, 125).

—— & HODGKIN, E. P. The *Anopheles umbrosus* group. In the press.

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ANAEMIAS OF AFRICANS IN KENYA *

BY
HENRY FOY
AND
ATHENA KONDI
(*Wellcome Trust Research Laboratories*),
A HARGREAVES
AND
J LOWRY
(*Colonial Medical Service, Kenya*)

Numerous authors have described anaemias among Africans, and have reported what they consider as either nutritional macrocytic anaemia, or simply macrocytic anaemia (ANDERSON, 1940, TROWELL, 1939, 1947, DICK and MCCARTHY, 1946, LEHMANN, 1949, BEET, 1949, etc)

A nutritional macrocytic anaemia is a fairly definite entity such as described in India and Macedonia The anaemias so far reported in Africans cannot be fitted into this picture (WILLS, 1932–38, FAIRLEY *et al*, 1938, FOY and KONDI, 1939)

In our opinion, a nutritional macrocytic, or a megaloblastic anaemia, in the sense that it occurs in India and Macedonia, must conform to the following pattern

(1) A high mean corpuscular volume (above $95\mu^3$) taken at a time when the reticulocytes are not above 1 per cent When they are between 5 and 15 per cent, then 1 cubic micron must be subtracted from the M C V for every 1 per cent rise in the reticulocytes

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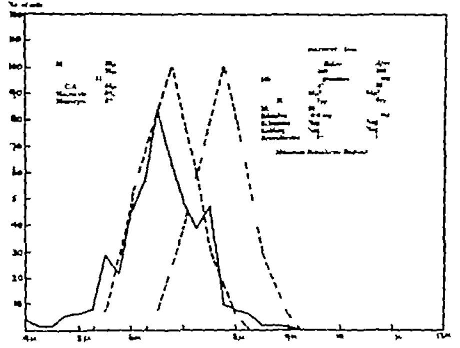
* We are greatly indebted to the Director of Medical Services, Kenya, for the facilities which he has given us to carry out these investigations, and for his permission to publish the results of the work To the Director of the Medical Research Laboratory for accommodation and innumerable other facilities without which none of this work would have been possible To Dr HARRIS, of the Kiambu Hospital, we are very much indebted for some of the cases that he kindly put at our complete disposal, and to Dr OTSYULA for cases in the Nairobi Group Hospital The European and African staff of the Nairobi Group Hospital have given us their unstinted aid throughout the whole course of the investigation, in spite of our many fastidious demands on them

If the reticulocytes are above 15 per cent. it is then necessary to determine the diameter distribution of the reticulocytes in order to calculate the percentage that are above the mean diameter so as to be able to make the correction in the M.C.V. Failure to observe this correction will lead to serious errors in diagnosis. (FOY and KONDI 1939 1943.)

GRAPH I. Macrocytic and Hyperchromic (no Megaloblasts and no Stab cells). Before Treatment.



TREATMENT—Iron.

	Before	After		Before	After
R.B.C.s	2·400.	5·450	M.C.H.C	23·6	19·4
Hb	5 3 gms. %	8·6 mg.	Bilirubin	0·6 mg.	0 5 mg
C.I	0·47	0·55	Schumms	Neg	Neg
M.C.V	56μ	$64\mu^3$	Sickling	Neg	Neg
M C.H.	18 $\gamma\gamma$	13·6$\gamma\gamma$	Reticulocytes	1·9	0 1

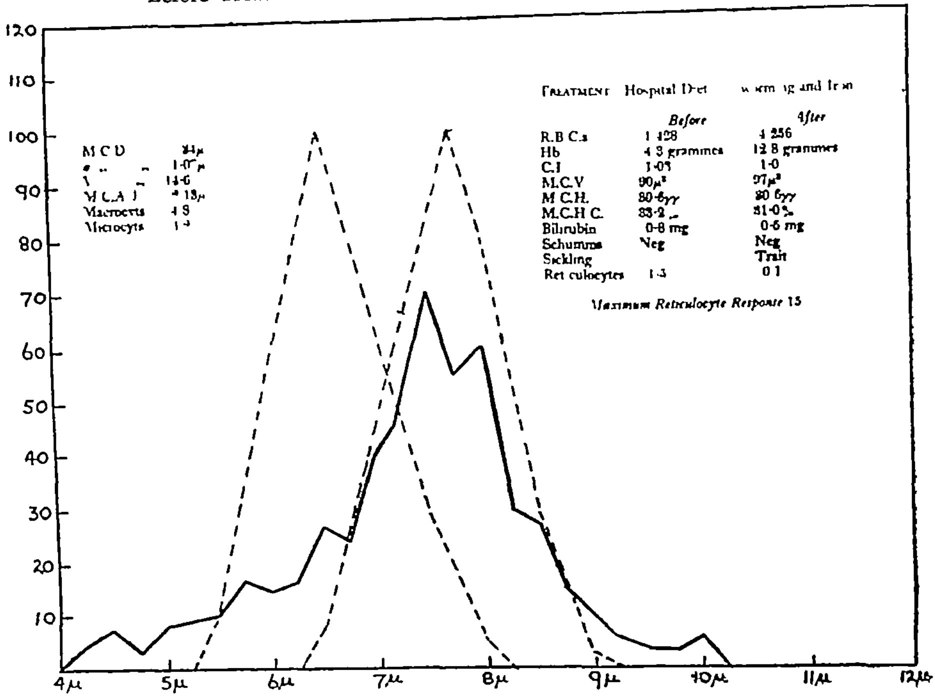
Maximum Reticulocyte Response 10

(*) High colour indices mean corpuscular haemoglobin concentrations, and mean corpuscular haemoglobins, calculated from haemoglobins done by some accurate method and blood cell counts done with an error of not more than ± 4 per cent. In cases where blood volume changes are suspected, these should be checked for by means of Evans Blue estimation of blood volume.

(3) The presence of Ehrlich megaloblasts and or giant stab-cells in the marrow and hypersegmented neutrophils in the peripheral blood. The former are characterized by finely netted "open" nuclei and "smooth" haemoglobinized cytoplasm, such as originally described by JONES (1934 1937) TURNBULL (1936) SCHULTEN (1937) and

ISRAELS (1930, 1939) They are characteristic of pernicious anaemia and the nutritional macrocytic anaemia of India and Macedonia They are pathological cells resulting from an upset in the maturation of the red cell series in the marrow due to lack of some factor present in liver principle They are not to be confused with the so-called " megaloblasts " of the American workers (SABIN, 1921 , DOAN, 1925), which are merely early erythroblasts and not to be regarded as pathological Considerable confusion has been, and still

GROUP II. Megaloblastic Normocytic and Normochromic [Megaloblasts and Stab cells] Before Treatment



TREATMENT—Hospital Diet, Worming and Iron

	Before	After		Before	After
R B Cs	1 428	4 256	M C H C	33 2%	31 0%
Hb	4 8 grms %	12 8 grms %	Bilirubin	0 8 mg %	0 5 mg %
C I	1 03	1 0	Schumms	Neg	Neg
M C V	$90\mu^3$	$97\mu^3$	Sickling	—	Trait
M C H	30 $6\gamma\gamma$	30 $6\gamma\gamma$	Reticulocytes	1 5%	0 1%

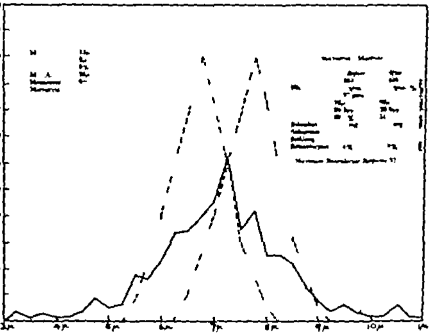
Maximum Reticulocyte Response 15%

is, caused in the literature by referring to early erythroblasts as megaloblasts Regarding the giant stab-cells (Riesen-Stabkern of Schulten, or giant metamyelocytes), these are also to be looked upon as pathological cells and are found in pernicious anaemia and nutritional macrocytic anaemias We regard these cells just as characteristic of these types of anaemia as are the megaloblasts of Ehrlich (FOY and KONDI, 1943 , FOY *et al* , 1946) Whether these giant stab-cells are the precursors of the hypersegmented neutrophils that are found in the peripheral blood is at present uncertain , it is possible that the latter represent a more advanced state in the development of the giant stab-cell

(4) The presence of free HCl in the gastric juice before or after histamine to exclude pernicious anaemia.

(5) A Price Jones curve taken at low reticulocyte level showing a shift to the right. We have used Leitz panphot with steady cadmium rays for all curves, and measured 500 cells. To avoid psychological selection (LARSEY 1945) all cells in the field should be measured.

GROUP II. Megaloblastic Normocytic (Megaloblasts and Stab-cell). Before Treatment.



TREATMENT—Marmite.

	Before	After		Before	After
R.B.Cs	1 831	3 415	M.C.H.C.	29·? %	1·8 %
Hb ..	8·8 gms.	9 gms.	Bilirubin	2·6 mg	0·8 mg %
Fe I	0·9? gms.	0·8	Schumm's	+	+
M.C.V.	85μ	63μ³	Sickling	—	—
M.C.H.	29 γγ	23·0γγ	Reticulocytes	1%	4·9%

Maximum Reticulocyte Response 87%.

(6) A response to known potent liver to marmite pteroylglutamic acid, or vitamin

(7) Data taken from pregnant women after they have given birth should be regarded with reserve so far as response to treatment is concerned since reticulocytes generally after the birth, especially if the child is not being breast fed.

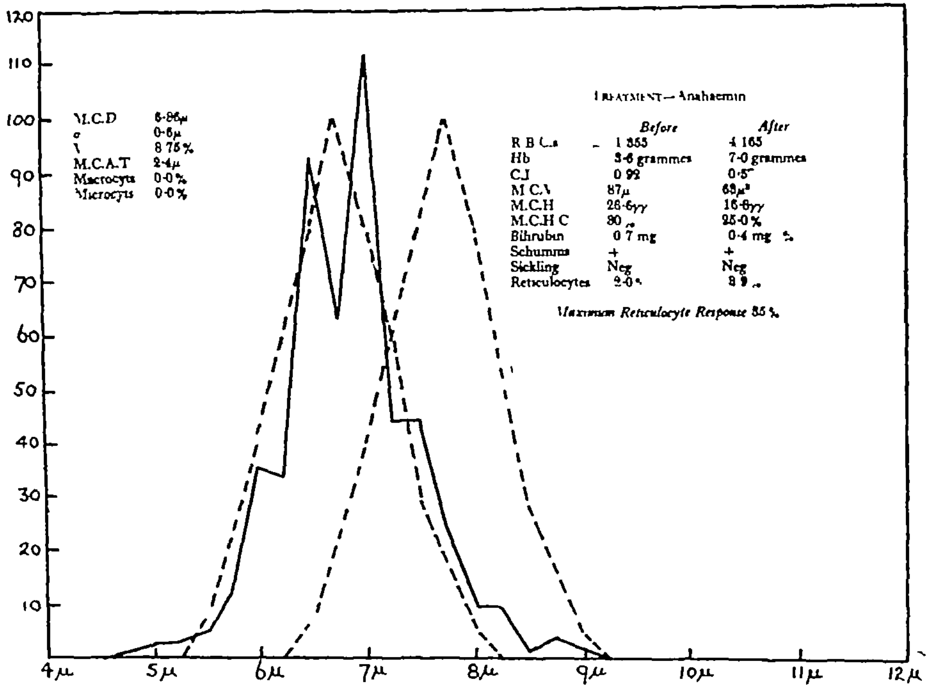
(8) Sickle-cell anaemia must be excluded in Africans.

(9) Cases should be under observation for 5 to 10 days and daily reticulocytes taken before any specific treatment is given.

The literature on African anaemias, so far as we are aware, nowhere conforms to these requirements, and without them no diagnosis of macrocytic or megaloblastic anaemia can, or should, be made

So far only one case of normocytic normochromic anaemia has been reported in a pregnant African with typical megaloblasts of Ehrlich in the bone marrow

GROUP III Normocytic Normochromic [Stab cells only] Before Treatment



TREATMENT—Anahaemin

	Before	After		Before	After
R.B C s	1 855	4 165	M C H C	30 %	25 0 %
Hb	8 6 grms %	7 0 grms %	Bilirubin	0 7 mg %	0 4 mg %
C I	0 92	0 57	Schumms	+	+
M C V	$87\mu^3$	$68\mu^3$	Sickling	Neg	Neg
M C H	$26\ 6\gamma\gamma$	$16\ 8\gamma\gamma$	Reticulocytes	2 0 %	3 9 %

Maximum Reticulocyte Response 35 %

This woman had a Price-Jones curve within the normal limits, indicating that a megaloblastic anaemia can occur which is not macrocytic (FOY and KONDI, 1943)

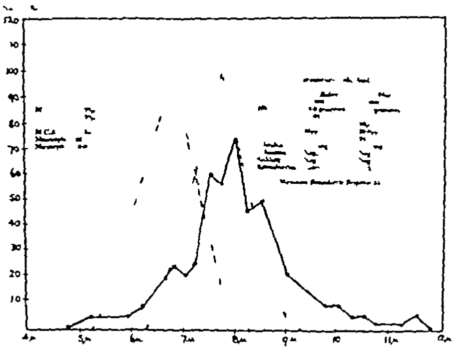
During the past 2 months we have had an opportunity of critically examining all the cases of anaemia that occur among Africans entering the Nairobi Group Hospital and from some of the outlying hospitals, and we are able to confirm that megaloblastic anaemias do occur among Africans that have not a

macrocytic blood picture, and which respond to either crude or refined liver to marmite or to pteroylglutamic acid.

The anaemia situation among Africans is undoubtedly complex, and from our investigations it appears that there are at least four different types of anaemia falling into the following categories

(1) Those with hypochromic macrocytic blood pictures with red cell counts of 1·5 millions or above They have neither megaloblasts nor giant stab-cells in the marrow

Group IV Megaloblastic Macrocytic (Megaloblasts and Stab-cells) Before Treatment.



Treatment—Folic Acid.

	Before	After		Before	After
R.B.C.s	0·990	4·000	M.C.H.C.	1%	24·4%
Hb	8·0 grms.	8·6 grms. %	Bilirubin	0·8 mg	0·4 mg
C.I.	1·01	0·7	Schumm	Neg	Neg
M.C.V. ..	122μ	83μ³	Sickling	Neg	Neg
M.C.H.	31γγ	20·8γγ	Reticulocytes	5·0%	0·2%

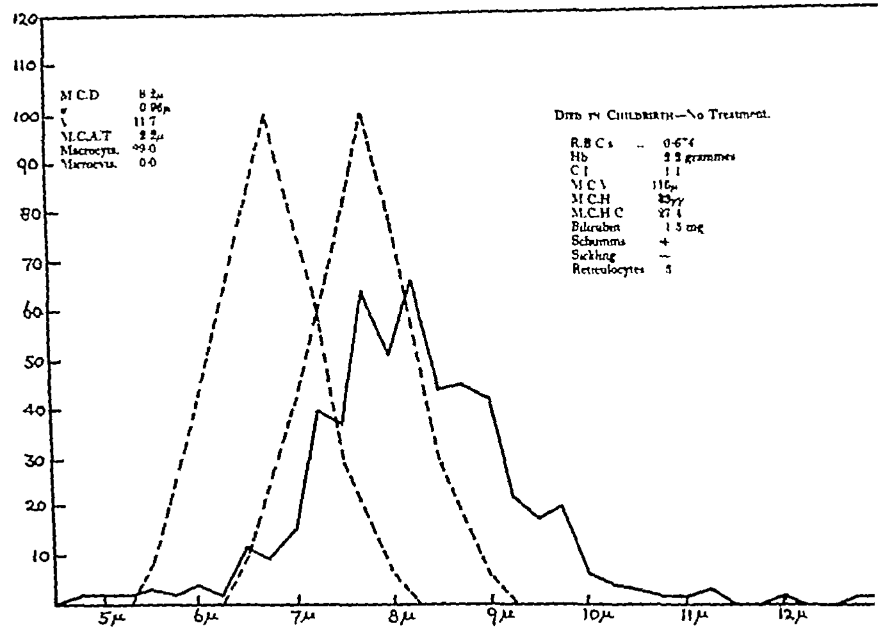
Maximum Reticulocyte Response 31%.

and there are no hypersegmented neutrophils in the peripheral blood. They have free acid in the gastric juice before histamine. The indirect van den Berg is never raised and Schumm test is negative They do not respond to either parenteral liver marmite or folic acid by mouth. The Price Jones curve is to the left of normal. They mostly or all have ancylostomes or schistosomes in the faeces, and they respond to worming and/or iron

therapy, and minimally to good hospital diet They are probably the classical worm anae
With worming, their red cell counts reach nearly normal levels, and their haemoglobir
concurrently Later, unless some iron therapy is given, the red cells outstri
haemoglobin production and the blood picture shows low M C H C and M C.H , v
colour index below 0 9 The reticulocytes response is that expected for the blood c

(2) Those having a normocytic normochromic blood picture with red cell c
between 1 0 and 1 5 millions There are typical Ehrlich's megaloblasts and giant
cells in the bone marrow and hypersegmented neutrophils in the peripheral blood

GROUP IV Megaloblastic Macrocytic [Megaloblasts and Stab-cells] Before Treatm



DIED IN CHILDBIRTH—No Treatment

R B Cs	0 674	M C H C	27 4 %
Hb	2 2 grammes %	Bilirubin	1 5 mg %
C I	1 1	Schumms	+
M C V	$116\mu^3$	Sickling	—
M C H	$38\gamma\gamma$	Reticulocytes	3 %

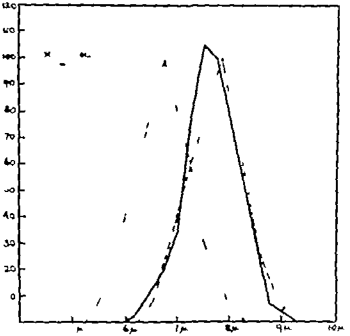
acid is present in the gastric juice before histamine , the Price-Jones curve is with
normal range The indirect van den Bergh is between 0 5 and 2 5 mg per
Schumm's test is sometimes positive The spleen may be enlarged, and oedema
legs may or may not be present These cases respond minimally to worming, h
diet, and maximally to crude or refined liver parenterally, pteroylglutamic acid or m
by mouth With any of the above treatments the blood counts rise to nearly normal
and the haemoglobin to about 10 0 grammes per cent , where it will tend to remain
iron therapy is given The failure of the haemoglobin to rise to higher levels n
due to inadequate worming, or to failure of the haemoglobin synthesis to keep pac
red cell production, owing to depleted iron stores With treatment the megal
disappear rapidly, the giant stab-cells very much more slowly

(3) Those with a normocytic normochromic blood picture with red cell
between 1 5 and 2 5 millions The marrow contains giant stab-cells but no megalo

the peripheral blood has hypersegmented neutrophils. The Price-Jones curve is within normal limits, and there is free acid in the gastric juice before histamine. The indirect van den Bergh is between 0·4 and 0·8 mg. per cent. and Schumm test may or may not be positive. Worms may be present. Sometimes there is oedema of the ankles and the spleen may be enlarged. These cases respond to the same treatment as given in the case of Group 2, but their haemoglobin does not tend to lag so much behind red cell production, probably on account of the lesser degree of anaemia.

(4) A group with characteristic macrocytic anaemias with high M.C.H., M.C.H.C. and high colour index. They have typical Ehrlich megaloblasts and giant stab-cells

NORMAL AFRIC N.



in the sternal marrow and hypersegmented neutrophils in the peripheral blood. The red cell counts are between 0·5 and 1 millions. The Price Jones curve is well to the right of normal and there is high percentage of macrocytosis. The indirect van den Bergh is between 0·0 and 4·0 mg. per cent. and Schumm test is positive. The gastric juice has free acid before and after histamine although there may be hypochlorhydria. The spleen may be enlarged and there is generally oedema of the legs, the degree depending on the anaemia. There is no glossitis or nervous involvement. They respond to crude or refined liver injections, or pteroylglutamic acid and to marmite. These cases re comparable in every respect to the Indian and Macedonian cases. They only differ from the Indian cases described by Wills in that the indirect van den Bergh is raised, and the response to anahaemin. Cases have however since been reported from Indians in whom the indirect van den Bergh is raised and who respond to anahaemin. We do not think that the raised van den Bergh and positive Schumm test is necessarily connected with malaria.

Nearly all the above subjects have intestinal parasites in the form of hook worms, schistosomes, *Entamoeba histolytica* and sometimes tapeworms. In

cases where they have not been found, the possibility that they have not been expelled by the vermifuge, or have been missed, even after multiple examinations for ova, should not be lost sight of

From this work it appears that a megaloblastic anaemia can exist in Africans that is not accompanied with a macrocytic blood picture as was previously reported (FOY and KONDI, 1943)

The megaloblastic macrocytic anaemias described here resemble those of India and Macedonia, where they have been attributed to a deficiency perhaps related to low intake of first-class protein Whether the megaloblastic macrocytic anaemias of Africans are similarly associated with dietary deficiency or imbalance is not yet known As there are African tribes here in Kenya which are said to live almost exclusively on non-meat diet (rural Kikuyu) and others which consume large amounts of first-class protein in the form of blood and meat (Masai), it should be possible here to determine the relation of diet to the occurrence of these megaloblastic macrocytic anaemias among Africans and to assess the place that first-class protein occupies in the picture

The exact part that intestinal worms play in the genesis and maintenance of these various types of anaemia is by no means clear Peripheral bleeding due to worms cannot be the only or main cause Worms are no doubt important so far as the microcytic hypochromic ones are concerned, that have low mean corpuscular volumes and colour indices, and Price-Jones curves to the left of normal But peripheral bleeding cannot account for those anaemias in which there are maturation defects in the marrow leading to the production of such pathological cells as Ehrlich's megaloblasts giant stab-cells and hypersegmented neutrophils The presence of these cells must indicate some disturbance of haemopoiesis that cannot be accounted for by peripheral bleeding alone The maturation defect may be due to a failure to synthesize, absorb or utilize haemopoietic factors consequent on worm infection, such as is the case in *Dibothriocephalus latus* (BONSDORFF, 1948) On the other hand, it may be due to direct dietary deficiency such as is the case in India and Macedonia, in the latter of which neither schistosomes nor hookworms are present The fact that some response can be obtained in the African cases to good hospital diet may indicate that food is an important factor A combination of diet and worms should not be lost sight of We do not consider it justifiable at present to disregard diet as a factor and perhaps a very important one, as in India and Macedonia

CAMERON *et al* (1949) have suggested that in the megaloblastic macrocytic anaemias of intestinal stricture there may be a change in the flora of the intestine that either (1) leads to loss of an organism that synthesizes haemopoietic factors, or (2) production of an organism that uses up the haemopoietic factors, or (3) production of an antagonist It may well be, as we have suggested above, that the intestinal worms present in our African cases are acting in a similar way BARKER and HUMMEL (1939) have put forward the view that in the megaloblastic macrocytic anaemias of intestinal stricture the stagnation that occurs

prevents haemopoietic factors from detoxifying substances in the intestine that depress the bone marrow function. If these and our views are correct it would appear that in the African cases intestinal disturbances are affecting the white and red cell maturation separately as we have pointed out above.

The fact that these subjects when they enter hospital show no reticulocytosis although they have no doubt been ill for long periods, may point to a depression of erythropoiesis which, together with the pathological cells in the marrow and peripheral blood, may indicate some toxic factor that is depressing marrow function.

Our view would be that both worms and dietary deficiencies are playing a part in African anaemias, and that a correction of both defects is necessary as suggested by TROWELL and LITHMANN. Peripheral bleeding and dietary imbalance are no doubt important in the low haemoglobin picture.

So far no quantitative worm counts have been done on whole faeces here but the impression of most workers is that there is not always a positive correlation between the degree of worm infestation and the magnitude of the anaemia (c.f. BEET 1949; DICK and MCCARTHY 1948). No doubt worms may play a role in the genesis of the anaemias in Africans, but whether it is a direct one due to peripheral bleeding or an indirect one interfering with production and utilization of haemopoietic factors, is by no means certain. The presence of maturation upsets in the marrow shown by presence of pathological cells would seem to us to indicate that peripheral bleeding is less important than other unknown factors. Here again Kenya offers a field for the examination of this problem since there are areas where hookworm infestation is very high, and others where it is absent or very light. Further there are said to be areas where the predominant worm is *Ascaris*, and where hookworm is comparatively rare. The exact place that malaria occupies in the picture is also possible of examination since there are regions where malaria is very common, and others where it is negligible.

The cases of megaloblastic anaemia here respond maximally to both refined and crude liver as well as to marmite and folic acid. The cases in Macedonia likewise respond to crude or refined liver given in large doses, to marmite and to folic acid. In Macedonia, 6 c.c. to 12 c.c. of campolon daily were necessary to get a response; the dose of anahaemin or procythol forte (churoin) 2 c.c. daily (FAIRLEY *et al.* 1938; FOY and KONDI 1939). WILLS reported that her Indian cases responded to marmite but not refined liver preparations such as anahaemin. Indian cases have since been found to respond to anahaemin and thus the situation in India and Macedonia brought into line (FAIRLEY 1940; NAPIER, 1938; SUNDARAN 1944–49; PATEL, 1949). It can now be stated that the nutritional megaloblastic macrocytic anaemias of both India and Macedonia respond to both the crude and refined liver extracts, to marmite, pteroylglutamic acid and vitamin B_{12}. So far as we have been able to ascertain at present, the African cases give similar responses to treatment. Whether they are similarly connected with dietary deficiencies remains to be seen.

The failure of haemoglobin to keep pace with red cell production, as mentioned above, is very striking in the African cases, it was not observed in the Macedonian cases Whether this is due to iron deficiency in the diet or to depletion of iron stores as a result of worm infection is at present not known In these circumstances it is well to give some form of iron therapy to correct this defect, as well as liver to amend the maturation upset in the marrow In a large proportion of our cases we have found that the response to iron ammonium citrate in doses of gramme 4 to 6 daily is unsatisfactory unless hydrochloric acid is given In these cases we have found that there was always very greatly reduced free and total acid in the gastric juice both before and after histamine

The existence of giant stab-cells in the sternal marrow and hypersegmented neutrophils in the peripheral blood but no megaloblasts, such as occurs in some of the African cases (Group 3), may indicate that there are two factors in liver principle One controlling the maturation of the red cell series, the absence of which results in the production of the pathological Ehrlich's megaloblasts, the other concerned with the maturation of the white cell line, the absence of which results in the production of the giant stab-cells These giant stab-cells are just as significant, in our opinion, as are Ehrlich's megaloblasts They are found in pernicious anaemia as well as the nutritional macrocytic megaloblastic anaemias of Macedonia The response to liver shown by these giant stab-cells, as pointed out below, is much slower than that of the megaloblasts, the latter disappearing very rapidly after the commencement of liver therapy (FOY and KONDI, 1943–46) If this view is correct there would appear to be cases in which the red cell maturation factor is absent, others in which the white cell factor is absent, and others in which both are lacking as shown in the cases reported above We have never found megaloblasts in the marrow without their being associated with giant stab-cells, but it is very common to find stab-cells without megaloblasts

Hitherto it has been customary to regard haemopoietic responses as a whole, but with the production of such specific factors as pteroylglutamic acid and vitamin B_{12} it should be possible to ascertain whether there are in fact several different factors which control the maturation of the red and white cell series separately The fact that pteroylglutamic acid given in pernicious anaemia will bring about a rapid blood regeneration but leave the nervous symptoms untouched or exaccerbate them, whilst liver treatment will relieve both the haemotological and neurological conditions, makes it appear as though folic acid contains only a "blood regenerating principle," whilst liver contains a "haemopoietic and neurotrophic principle" It appears from the work of UNGLEY (1949) that B_{12} is also effective in controlling the neurological symptoms We are aware that the question of "antagonist" enters into this problem (JUKES, 1948, FILDES *et al*, 1949), but feel that the time has come to consider the possibility of a red and white cell maturation factor acting separately MAY *et al* (1941) have found that in the megaloblastic macrocytic anaemias produced

in monkeys by deficient diet, the marrow defects can be corrected in the early stages of the disease by the administration of ascorbic acid and folic acid, but that B_{12} has no effect on the marrow in 48 hours.

The presence of Ehrlich's megaloblasts in conjunction with anaemias that have normal mean corpuscular volumes and Price-Jones curves within the normal range raises the question of the fate of the megaloblasts and the origin of macrocytosis. It is customary to regard the megaloblasts as the precursor of the macrocyte but the cases here and the one previously reported make it quite certain that a megaloblastic anaemia can occur that is not macrocytic. In certain liver diseases macrocytic anaemia occurs, but there are no Ehrlich's megaloblasts or other pathological cells in the marrow indicating that the cell maturation factor is present, but the cell diameters are increased. Is it possible that there may be a third factor present in liver principle that is controlling cell diameters, the interference with which results in the production of cells with an abnormal diameter or thickness? We are well aware that there are innumerable factors concerned with the regulation of cell diameters (PONDER, 1934, 1948; KNOOP, 1946) but the frequent association of megaloblasts and macrocytosis and its absence in these African cases leads to the consideration of the existence of a diameter regulating factor in liver principle. So far the only normocytic megaloblastic anaemias in man that have been reported have been in Africans in which worm infection is invariably present. It is not impossible that a combination of worm infection and dietary deficiency may be responsible for this peculiar condition. We have never seen in Macedonia a megaloblastic anaemia that is not also macrocytic and we are unaware of any other report of such a condition (SUNDARAM, 1949).

In the case of the macrocytic anaemias the Price-Jones curves show great anisocytosis and the three cell populations that are characteristic of pernicious anaemia before treatment. Further details concerning cell size distribution in the African cases will be published later.

Work is continuing on these four types of anaemia and a full report, together with critical results of treatment on the Price-Jones curves, marrows and peripheral blood, will be published later. Below we are merely outlining representatives of the four types with brief notes on the response to the various treatments given.

SUMMARY

1. A survey has been made of the anaemias occurring in Africans in Kenya, and certain criteria laid down for their proper diagnosis. The macrocytic anaemias so far reported from Africa have turned out to be due to reticulocytosis and not macrocytic in the usual meaning of that term.

2. The anaemias in Africans in Kenya fall into at least four fairly sharply defined groups.

3. In three of the groups Ehrlich's megaloblasts and/or giant stab-cells are present in the marrow, and hypersegmented neutrophils in the peripheral

blood The presence of these pathological cells clearly indicates that peripheral bleeding cannot be the only or even the most important factor involved in the genesis and maintenance of these anaemias

4 There must obviously be some haemopoietic disturbance which results in a maturation failure resulting in the development of megaloblasts and giant stab-cells Whether this haemopoietic disturbance is due to direct nutritional deficiency as in India and Macedonia is not yet certain It is not impossible that worm infestation may interfere with the synthesis, absorption or utilization of haemopoietic factors as in the case of *Dibothriocephalus latus* infestation, in which cases peripheral bleeding due to the worms may be an additional complication

5 The absence of reticulocytosis, and the presence of these pathological cells in untreated African cases, indicate both a depression of erythropoiesis and abnormal maturation that may be due to toxic factors connected with worm infestation and/or dietary deficiencies

6 All those anaemias here which have pathological red and white cells in the marrow respond maximally to refined or crude liver extracts inter-parenterally, to marmite or folic acid by mouth, and more slowly to good hospital diet

7 The rapid response of red cells to liver, marmite or folic acid outstrips the haemoglobin production, and unless iron therapy is given a hypochromic blood picture will develop This failure of the haemoglobin to keep pace with red cell production may be due to depleted iron stores consequent on low iron content of the diet, to worms, or to both It is recommended that iron therapy be given to correct lag in haemoglobin and haemopoietic substances to amend the maturation defect in the marrow

8 The existence of a megaloblastic anaemia that is not macrocytic has been confirmed among Africans, and the problem of the fate of the megaloblast and the origin of macrocytosis is discussed

9 The presence of anaemias with both Ehrlich's megaloblasts and giant stab-cells, and others that have only giant stab-cells, suggests (1) that there may be red and white cell maturation factors in liver principle that are acting separately, or (2) that a profound deficiency produces upsets in both white and red cell maturation, a lesser deficiency affecting only the white cell line Folic acid would appear to have only a " blood regenerating factor " since it leaves the nervous system of pernicious anaemia untouched Liver treats both the blood and neurological symptoms of pernicious anaemia

10 The association of a megaloblastic marrow with a normocytic normochromic blood picture may indicate that there is a third factor in liver principle controlling cell diameters In liver disease macrocytosis occurs without megaloblasts

11 All the cases have free acid in the gastric juice before or after histamine, but hypochlorhydria is common , two of the groups have raised indirect van den Bergh and positive Schumm's tests

12. The response that three of the four groups of these anaemias give to marmite and the readiness with which the African takes to this substance suggests that this might form a valuable dietary supplement in areas where diet is inadequate.

13. The Price Jones curves are within normal limits in two of the groups—to the left in the microcytic hypochromic "worm" anaemias and to the right in the megaloblastic macrocytic ones.

REFERENCES

ANDERSON, T. F. & ROBERTS, J. I. (1940). *Trans. R. Soc. trop. Med. Hyg.*, 33, 615.
B[illegible], W. H. & H[illegible], L. E. (1939). *Johns Hopk. Hosp. Bull.*, 64, 15.
BITT, E. A. (1949). *Trans. R. Soc. trop. Med. Hyg.*, 43, 317.
CAMERON, D. G., WATSON, G. M. & WITTS, L. G. (1949). *Blood*, 4 (7), 793.
DICK, G. W. & MCCARTHY, D. D. (1946). *E. Afr. med. J.*, 23, 19.
DOAN, C. A., CUNNINGHAM, R. S. & SABIN, F. R. (1925). *Contr. Embryol. Carneg. Inst.*, 16, 163.
FILDES, P., WOODS, D. D., MCILWAIN, H., *et al.* (1948). *Proc. roy. Soc. B*, 135, 833, 145.
FAIRLEY, N. H., BROMFIELD, R. J., FOY, H., KONDI, A. (1938). *Trans. R. Soc. trop. Med. Hyg.*, 32, 132.
——— (1940). *Lancet*, 1, 1118.
FOY, H. & KONDI, A. (1938). *Ibid.*, 2, 360.
——— ——— (1943). *Ibid.*, 2, 506.
——— ——— & MURRAY, J. F. (1946). *J. Path. Bact.*, 58, 157.
ISRAELS, M. C. G. (1930). *Ibid.*, 49, 231.
——— (1939). *Ibid.*, 52, 17.
JONES, O. P. (1937). *Arch. int. Med.*, 60, 1002.
——— (1934). *Anat. Rec.* (Suppl.), 58, 23.
JUKES, J. H. & STOKSTAD, E. L. P. (1948). *Physiol. Rev.*, 28, 51.
KROGH, A. (1946). *Proc. roy. Soc. B*, 133, 140.
LARSEN, E. (1948). *Distribution of Red Blood Cell Diameters in Liver Disease.* Oslo.
LEHMANN, H. (1949). *Lancet*, 1, 90.
——— (1949). *Nature*, 164, 954.
NAPIER, L. E., *et al.* (1938). *Indian Med. Gaz.*, 73, 385.
PATEL, J. C. & BHENDE, Y. M. (1949). *Blood*, 4 (3), 259.
PONDER, E. (1934). *The Mammalian Red Cell and the Properties of Haemolytic Systems.* Berlin: Verlag Gebruder Borntraeger.
——— (1948). *Haemolysis and Related Phenomena.* New York: Grune Stratton.
SABIN, F. R. (1921). *Johns Hopk. Hosp. Bull.*, 32, 314.
SCHULTEN, H. (1937). *Die Sternalpunktion Als Diagnostische Methode.* Leipzig: Thieme.
SUNDARAM, S. K. (1948). *Lancet*, 1, 568.
TROWELL, H. C. (1939). *E. Afr. med. J.*, 16, 12, 402.
——— (1940). *Ibid.*, 17, 14.
——— (1947). *Lancet*, 1, 43.
——— (1943). *Trans. R. Soc. trop. Med. Hyg.*, 37, 19.
——— (1947). *S. Af. J. med. Sci.*, 12, 1, 21.
TURNBULL, H. N. (1936). [illegible] Vaughan, *Anaemias.*
UNGLEY, C. C. (1949). *Brit. med. J.*, 2, 1370.
WILLS, L. & EVANS, B. D. (1934). *Lancet*, 2, 416.
——— MEHTA, M., TALPADE & BILIMORIA, H. S. (1930). *Indian J. med. Res.*, 17, 777.
——— ——— ——— & ——— (1930). *Ibid.*, 18, 253.
——— ——— ——— & ——— (1932). *Ibid.*, 20, 391.
——— ——— ——— & ——— (1934). *Ibid.*, 21, 669.

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THE LIVER IN RELATION TO PROTOZOAL INFECTIONS *

BY
P C C GARNHAM
Reader in Medical Parasitology, University of London

I thought that before writing this paper I should consult some standard works on the liver and its diseases , what was my surprise when I found that not a single word in these books referred to protozoal diseases, in spite of the fact that malaria, for instance, is the greatest single cause of morbidity in the human race , so perhaps this approach to the subject is not inopportune I do not intend to discuss, this evening, all the Protozoa which affect the liver , in fact, I am excluding nearly all the organisms which cause extensive damage to its substance, such as *Entamoeba histolytica* which gives rise to amoebic abscesses, or *Histomonas meleagridis* producing blackhead in turkeys or *Leishmania donovani*, the cause of kala-azar The group of Protozoa that I am going to refer to are the Sporozoa, the class which includes the malaria parasites Recent work on the life history of these organisms—chiefly by the President of our Society—has brought the liver very much into the limelight First I must say a little about the organ itself

The liver is the largest gland in the body and is indispensable to life It is composed of lobules about 2 mm in diameter, each lobule consisting of columns of parenchyma cells Around each lobule is connective tissue wherein travel branches of the portal vein, hepatic artery and bile duct. In the centre of each lobule is a branch of the hepatic vein The two circulations meet in the sinusoids between the columns of liver cells The sinusoids are lined by the strongly phagocytic Kupffer cells and ordinary endothelium , it is improbable that the blood comes into direct contact with the parenchyma itself

The liver develops as a ventral outgrowth of the endoderm of the duodenal portion of the mid-gut It projects itself into a mass of undifferentiated mesoderm via which it becomes vascularized and from which it obtains its connective tissue framework These two elements—the mid-gut endoderm and

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the mesoderm—are later to become the seat of two very distinct types of invasion by Protozoa. Protozoa develop in at least four types of cells in the liver—in the bile duct epithelium, in the Kupffer cells in the vascular endothelium and in the parenchyma cells. These last are perhaps the most interesting they are polygonal in shape with a large nucleus containing a prominent nucleolus. Binucleate cells are quite common. They have many functions, including the formation of glycogen and the synthesis of amino-acids in protein metabolism. Their protoplasm is thus very rich in foodstuffs probably richer and more varied in this respect than any other cell of the body Some of the Protozoa which grow in these cells need enormous quantities of nucleo-protein for their chromatin and it is easy to understand why liver cells provide the only suitable environment for the development of such organisms

The first infection I shall mention is that produced by the Coccidia which are usually parasites of the intestinal epithelium and give rise to a diarrhoea which is often fatal (*e g* the well known grouse disease of Scotland or the more benign *Isospora* infection in man) In the case of *Eimeria stiedae* a coccidian of the rabbit the sporozoites set free from the oocyst in the small intestine go to the epithelium of the bile ducts in the liver instead of to intestinal cells. They were originally thought to reach the liver via the bile ducts SMETANA (1933), however has shown that they travel by the portal vein. The terminal branches of the portal vein form a plexus surrounding the branches of the bile ducts (ANDREWS, MAEGRAITH and WENYON 1949), so a sporozoite in the vein has easy access to the epithelium of the duct. The parasites multiply in the epithelium of the bile ducts which become enormously hypertrophied to form characteristic arborescent tumours. These occupy much of the liver substance and the rabbits and hares which suffer from this infection sicken and die. The tumour substance is composed of adenomata derived from the epithelium of the bile duct. The cells of the tumour tissue contain parasites in all stages of development. Of all the sporozoan parasites that we are going to consider this is the only one that gives rise to tumour formation. It requires the bile duct epithelium to grow in it has not learnt—as most of the others have—how to live peacefully in its hepatic surroundings it multiplies in a malignant way and eventually destroys its host.

The other parasites as a rule are more successful. In the closely related family of haemogregarines there is an organism *Hepatozoon* common in the rat, which undergoes its asexual development in neat little schizonts inside the parenchymatous cells of the liver This parasite is usually satisfied with a few of these cells and causes so little disturbance to the host that even when the schizont ruptures, there is such a small quantity of toxin produced that no phagocytes appear on the scene The schizont ruptures and sets free gametocytes or haemogregarines which become lodged in the leucocytes and circulate in the blood until the animal is bitten by a mite in which the gametocytes undergo further development. The mite is eaten by another rat, the sporozoites

are set free by digestion in the intestine and they travel by the portal vein to the liver In the laboratory-bred white mouse, the *Hepatozoon* may cause more trouble than in wild animals—the liver becomes so riddled with the parasites that a severe disease is caused, the mice become extremely anaemic and die within 4 days (MILLER, 1908, BRUMPT, 1946)

Let us now turn to the Haemosporidiidea—the sub-order which includes the malaria parasite In the less highly specialized genera, the organisms multiply in various organs and tissues of the body, utilizing the endothelium In the case of *Haemoproteus*—a very common and widespread infection of birds—the parasite undergoes its asexual development in the endothelium of blood vessels, and in some species the capillaries of the liver are particularly selected The parasite becomes lodged in the endothelial cell of a capillary, rapid growth occurs and multinucleate bodies gradually spread up and down the capillaries and block the lumen

Another bird parasite, *Plasmodium gallinaceum*, chooses the Kupffer cells lining the blood sinusoids for its exo-erythrocytic schizogony How much harm is caused by the liver infection in bird malaria is unknown The massive infiltration of the capillaries of the brain is undoubtedly more important in causing death than is the infection in the liver

The bats of Palestine are infected with malarial parasites, which also undergo exo-erythrocytic development in the endothelial cells of the liver, but this time, they attack the undifferentiated lining cells of the sinusoids rather than the Kupffer cells Such cells are small and the schizonts which grow in them are no larger than 6μ There is no apparent host reaction and the bats seem to live quite happily with their parasites—which emerge into the blood as gametocytes waiting to be taken up by some as yet undiscovered vector

We are gradually approaching the human malaria parasites, but before reaching them we must study two organisms, *Leucocytozoon* and *Hepatocystes*, whose life-cycles foreshadowed the pre-erythrocytic development of the Plasmodia of man So far, most of the parasites which we have been considering have been minute and quite invisible to the unaided eye In the remainder the liver forms are much larger, indeed, in *Hepatocystes* they are 2 mm in diameter

Leucocytozoon is a blood parasite of many kinds of birds throughout the world It is transmitted by the *Simulium* fly and it sometimes causes severe outbreaks of disease—flocks of turkeys are very susceptible and may be entirely destroyed by the infection I have often picked up young nestlings of weaver birds dying from the disease, if the liver (or spleen) of such birds is sectioned, a most extraordinary appearance is revealed The parasite develops to form megalo-schizonts inside the parenchyma cells, or in fixed or wandering histiocytes (HUFF, 1942, WINGSTRAND, 1948) As growth proceeds the parasite becomes divided into numerous compartments or cytomeres which finally give rise to the merozoites Meanwhile the most striking change is going on in the host cell This cell expands in size to accommodate the growing parasite

which is eventually nearly half a millimetre in diameter. The cell nucleus undergoes a sort of gigantism—finally reaching a diameter of 190μ surrounded by the cytomeres of the *Leucocytozoon*. It looks as though the invader must secrete some growth-stimulating substance to cause this transformation. The *nucleus contains large quantities of chromatin distributed* around the nuclear membrane and in strands or irregular blocks throughout the interior. These megalo-schizonts are found not only in the liver but also in the spleen, pancreas and ductless glands.

The very common malaria parasite of African monkeys, *H. kochi* undergoes a rather similar process of development (GARNHAM, 1948) but this time it is confined to the parenchyma cells of the liver. Beginning as a minute body in the cytoplasm, it grows rapidly into a multi-lobular structure with many nuclei. The parenchyma cell becomes enlarged and the nucleus divides, eventually into eight or more nuclei jammed between the parasite and the remains of the cell wall. These nuclei are larger than normal and in later stages become pyknotic. The parasite seems to stimulate an abnormal growth of the cell which expands rapidly leaving a clear area between the parasite and the host cell wall. The clear area may be due to shrinkage during fixation. Vacuolation of the organism follows, the vacuoles coalesce and the nuclei collect in countless numbers in a thick rim of cytoplasm around a large vacuole. This merocyst on maturity measures 2 mm. in diameter and is visible to the naked eye as a tiny transparent body on the surface of the liver. Hundreds of thousands, if not millions, of merozoites are produced by each merocyst, so only a few cysts are necessary to maintain an infection; as a rule 6 to 12 at most can be found on the surface of the organ. We shall see the significance of this a little later.

TYPES OF DEVELOPMENT OF SPOROZOAN PARASITES IN LIVER
(Semi-diagrammatic).

PANEL 1.—*Plasmodium cynomolgi*. Pre-erythrocytic schizonts in parenchyma cells. Note enlargement of cell with growth of parasite.

PANEL 2.—*Plasmodium gallinaceum*. Exo-erythrocytic schizonts in Kupffer cells.

PANEL 3.—Malaria parasite of Palestine bats (MER and GOLDBLUM, 1947). Exo-erythrocytic schizonts in lining endothelial cells. Note pseudo-cytomere effect of largest group.

PANEL 4.—*Haemoproteus* sp. Young parasites in lining endothelial cells (after WENYON). Large form with seven divisions.

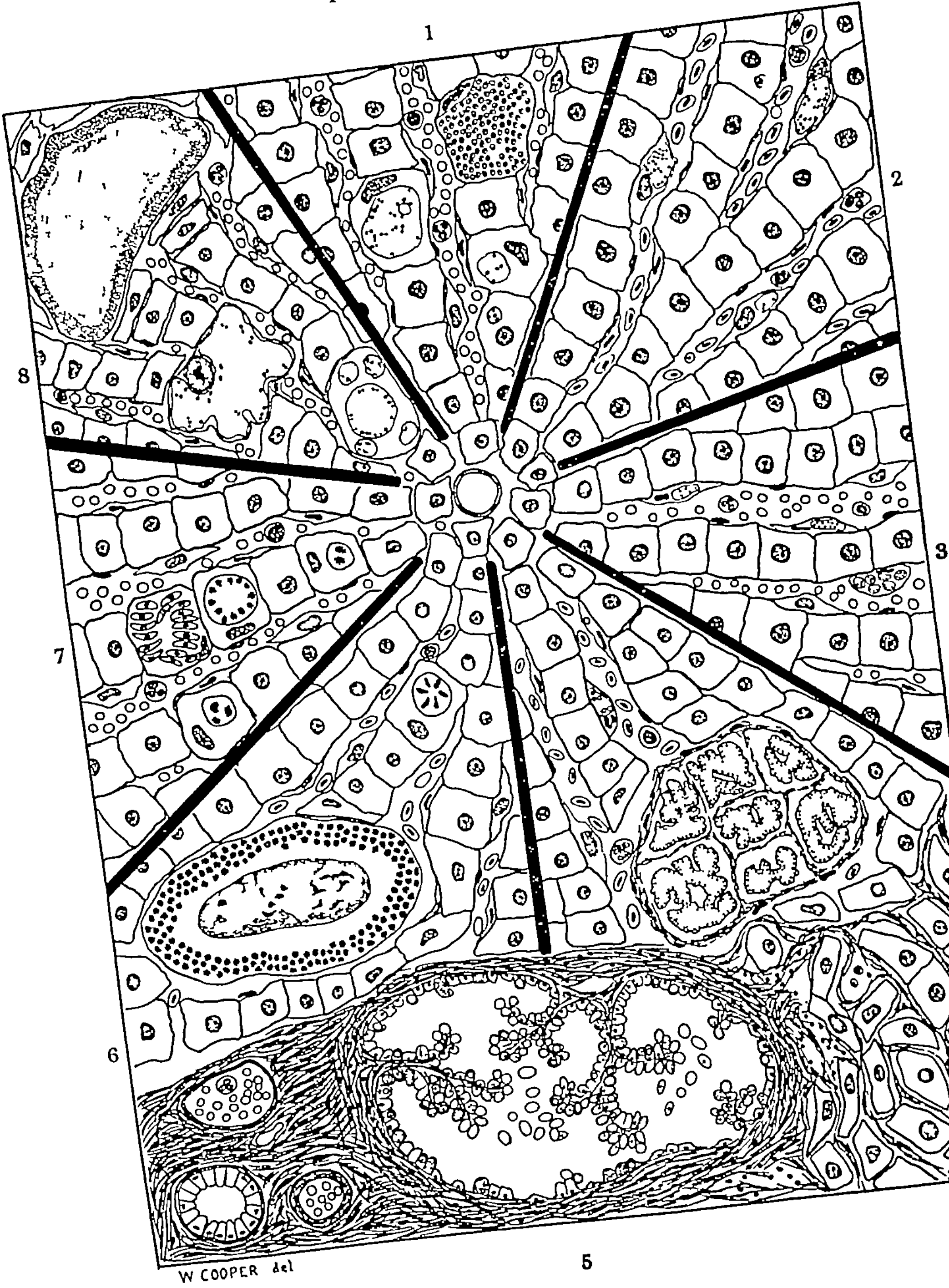
PANEL 5.—*Eimeria stiedae*. Infiltration of bile duct epithelium leading to arborescent hypertrophy. Note loose oocysts, and asexual and sexual forms inside epithelial cells.

PANEL 6.—*Leucocytozoon* sp. Early form in parenchyma cell (after HUFF, 1942) and nearly mature form showing cytomeres surrounding enormously hypertrophied host cell nucleus (from material kindly supplied by WINGSTRAND).

PANEL 7.—*Hepatozoon* sp. Growth of parasite in parenchyma cell.

PANEL 8.—*Hepatocystis kochi*. Early form in much enlarged parenchyma cell with multiple nuclei, later stage showing enlarged host cell nucleus and mature merocyst.

P C C GARNHAM



The merozoites escape into the blood and grow into spherical gametocytes in the red blood cells

During the growth of both *Leucocytozoon* and *H kochi*, there is absolutely no cellular reaction around the parasites In spite of their size, they appear to be quite innocuous With the maturity of the *H kochi* merocyst a zone of reaction forms around it First polymorphs accumulate and rarely succeed in making their way into the interior of the cyst More usually the thick wall resists penetration and the polymorph attack subsides, to be replaced by a more prolonged onslaught by lymphoid macrophage cells Very large giant cells accumulate around the parasite and also cells of doubtful origin containing eosinophile granules After rupture, all these cells play a part in clearing up the débris Finally, fibroblastic transformation occurs and an opaque scar is all that remains

Another malaria parasite, *H vassali*, undergoes practically the same sort of development in the liver of Malabar squirrels (FIELD and EDESON, 1949) In the flying squirrel of the Himalayas, again the same type of schizogony is seen, though here the cysts are sometimes multiple (RAY, 1949)

Finally, we come to the human malaria parasites and their habitat in the liver Reticulo-endothelial activity inside this organ in malaria has long been recognized The Kupffer cells multiply and hypertrophy as the disease becomes established , they phagocytose free merozoites, pigment, infected erythrocytes, cellular débris and everything that comes their way In really active states of premunition, the endothelial lining of the sinusoids becomes so swollen that the circulation of the blood is slowed down, and a condition of anoxia results The Liverpool School (*e g* , MAEGRAITH, 1948) ascribe the anoxia more to constriction of the hepatic veins than to actual blockage The parasites in the meantime have been multiplying in the blood vessels and when there is any stasis as on the hepatic vein side of the circulation, *Plasmodium falciparum*, the parasite of malignant tertian malaria grows into mature schizonts in the red blood cells I will not linger over this stage of the human disease , instead, I will pass on to the tissue phase of the parasite within the liver

The sporozoites inoculated by the bite of a mosquito reach the blood stream either by direct penetration of a capillary or via the lymphatics and the thoracic duct (LLOYD and SOMERVILLE, 1949) They remain in the blood for not more than half an hour and then disappear (FAIRLEY, 1945) They must find temporary accommodation somewhere in the liver, for this is where they are seen 4 days later, and the blood stream in the meantime has not become infective If development were occurring in, say, the spleen, and a preliminary generation of parasites were discharged into the circulation for transfer to the liver, then the blood should become temporarily re-infective at some time during these 4 days The blood, however, remains uniformly sterile, so it must be assumed that the liver is the primary site of development What happens during the first 4 days in this organ is still uncertain Material examined during this period so far has either shown no parasites or bodies of an indeterminate

nature. There appear to be two possible courses open to the sporozoite The German workers (MUDROW KIKUTH, 1949) think that the sporozoites undergo their first development in reticulo-endothelial cells in the liver and that a small number of merozoites are produced which subsequently invade the parenchyma. This theory would explain the rapid disappearance, by phagocytosis of sporozoites from the blood stream. The alternative idea that the sporozoites whilst travelling in the slow current of blood in the sinusoids, step off and penetrate the liver cells directly seems less probable. The parenchyma is not in direct contact with the blood, and it is not phagocytic, so it would be difficult to explain the rapid absorption of the sporozoites on this basis.

It is not until 4 days after the introduction of the sporozoites that developmental forms have, as yet, been detected. The first half of this period might be occupied by growth in reticulo-endothelial cells, and the second half in parenchyma cells where the parasites are first seen as oval or round bodies, 8μ in diameter with 24 nuclei. American workers (*e g* COULSTON, 1949) have introduced sporozoites directly into the liver and actually claim to have seen developmental stages in R.E. cells.

By the fourth day the organism occupies about a quarter of a parenchyma cell this has already become slightly enlarged and its nucleus has been pushed to one side. The parasite then grows very rapidly and by the seventh day in the case of *P vivax*, reaches a maximum size of 42μ in diameter On maturity it contains a thousand or more merozoites which invade the blood stream and start the well-known cycle.

During the growth of the parasite, the liver cell increases in size, the nucleus becomes flattened, and finally little trace of the cell itself remains. There is no sign of any reaction until schizogony is complete and rupture has occurred. Then immediate invasion of the area takes place, chiefly by lymphoid macrophage cells but also by a small number of polymorphonuclears. These cells phagocytose both the débris formed by the ruptured parasite and host cell and also a greater or lesser number of merozoites (the number perhaps depending upon the degree of immunity possessed by the host). Focal infiltrations f such cells may be seen in sections of liver they can be distinguished from similar aggregations around portal tracts by being found in any part of the liver lobule. The schizonts apparently occur equally throughout the organ.

The process I have just described is known as pre-erythrocytic schizogony and it has been observed in the greatest detail in the monkey parasite *P cynomolgi* (SHORTT and GARNHAM, 1948), but also in *P vivax* the cause of benign tertian malaria in man. Recently this stage has been demonstrated in malignant tertian malaria (SHORTT *et al.*) where it assumes much the same form as the preceding The growth of the schizont in a parenchyma cell is, however more rapid and the body of the parasite tends to expand n any direction where the pressure from the liver tissue is least. Thus a multilobular rather than an oval body is eventually produced—over 60μ in length and, when mature,

containing more than 10,000 merozoites At the end of the fifth day these escape into the circulation and initiate the blood cycle

In the case of benign tertian malaria, it seems that a few of the merozoites of a liver schizont re-enter liver cells instead of going to the blood, and that an intra-hepatic cycle continues quite independently of the cycle in the peripheral blood The development of immunity in the host fails to affect the exo-erythrocytic parasites, which are also protected by their habitat from the effects of most anti-malarial drugs I do not know whether it is significant or not, but exo-erythrocytic or relapse forms seem to possess a thicker outer membrane than the thin cell wall of the pre-erythrocytic schizont Otherwise the morphology of the two appears to be identical

Experimental infections were produced by employing hundreds of mosquitoes, each of which was heavily infected with sporozoites In these circumstances, tissue parasites were readily found in sections of the liver—as many as 20 in a section Experimental human infections were not so prolific, because of the disproportion in size between liver and inoculum In natural infections, which have arisen as a rule by the bite of a single, not very heavily infected, mosquito, the number of pre-erythrocytic schizonts must be extremely small Hence it is very improbable that they will ever be found under natural conditions They could be found, of course, but the chances must be astronomically remote Because these schizonts are so few in number, it is necessary for them to contain very numerous merozoites in order to produce an infection In the case of *H kochi*, there are perhaps as few as a dozen in the liver, but each produces millions of merozoites which are enough for the maintenance of the infection Under these circumstances, where only a tiny proportion of the liver is involved, it is obvious that the organ will not show much response to the preliminary infection Liver function tests in the incubation period are unlikely to be abnormal, and cirrhotic changes (due to tissue parasites) are improbable later

Discussion

The richness of the liver tissue enables it to support a diversity of protozoal life not to be met with in any other organ of the body The liver is able to look after itself so well as a rule that the parasites seem to cause little harm Sometimes by sheer weight of numbers they disturb or destroy its functioning, and in the exceptional case of the rabbit coccidian, the bile ducts are invaded and tumour formation is the result Otherwise even when the parasite grows to a size visible to the naked eye, there is no reaction on the part of the host until rupture of the parasite occurs and the débris has to be got rid of Such happy relations are confined to the Sporozoa, I have not discussed the formidable lesions produced by *Entamoeba histolytica* and other parasites

I have traced the development of the liver from the endoderm of the mid-gut and have shown how the common coccidian parasites of the mid-gut

are represented also in the bile ducts of the adult liver HAWKING *et al.* (1948) have also emphasized the intestinal origin of this group and have indicated the evolutionary changes. The mesodermal elements of the liver are of two types, vascular and phagocytic both provide homes for their own particular parasites, the endothelium of blood vessels harbouring a malaria parasite of bats, whilst the Kupffer cells contain the malaria parasites of birds. All these cells are comparatively undifferentiated and are found in other organs besides the liver their respective parasites are equally widely distributed. It is otherwise with the highly specialized parenchyma cell. The human malaria parasite (and certain other forms) needs this for its development and consequently the tissue phase of these infections is confined to the liver

Although host reaction around the growing organism is notable by its absence, the situation is quite different in regard to the cell which has been invaded. BRUMPT (1949) has drawn attention to the considerable hypertrophy of tissue cells following invasion by Protozoa. The capacity of the liver cell to expand in order to contain the developing protozoon is prodigious. It does not merely act as a sac to surround the organism, but undergoes a remarkable form of growth itself The nucleus usually enlarges to some extent it may then either divide into a fairly large number of daughter nuclei, or undergo transformation into an oval body many thousands of times the volume of the original nucleus. In other cases, it becomes flattened against the cell membrane and disappears. The cell itself is still recognizable up to 150μ in diameter These changes appear to be unique in morbid cytology and are worth further study

REFERENCES

ANDREWS W H. H., MAEGRAITH, B G & WENYON C E. M (1949) *Ann trop Med Parasit.*, 43 229

BRUMPT E. (1946) *Ann. Parasit hum comp* 21 1

——— (1949). *Précis de Parasitologie* 6th Ed. Vol I Paris Masson et Cie

COULSTON F (1949) *Proc Soc exp. Biol* 70 390

FAIRLEY N H (1945) *Trans R. Soc trop Med Hyg* 38 311

FIELD, J & EDESON, J F B. (1949) *Ibid* 43, 589

GARNHAM, P C C (1948) *Ibid* 41 601

HAWKING, F PERRY W L. M & THURSTON J P (1948) *Lancet* 1 728

HUFF C. H (1942) *J infect. Dis* 71 18

LLOYD O C. & SOMERVILLE, T (1949). *J Path Bact* 61 144

MAEGRAITH B G (1948). *Pathological Processes in Malaria and Blackwater Fever* Oxford Blackwell Scientific Publications

MILLER, W W (1908) *U.S Hyg Lab Bull* 46, 5

MUDROW L & KRAUTH, W (1949) *Dtsch. med Wschr* 74 789

——— (1949). *Z. Trop Parasit* 1 113.

RAY H. N (1949). *Proc nat Inst Sci., India,* 15 41

SHORTT H E. & GARNHAM, P C C. (1948) *Trans. R Soc trop Med. Hyg* 41 785

——— FAIRLEY N H., COVELL, G SHUTE, P G & GARNHAM, P C C (1949) *Brit med J* 2, 1006

SMETANA, H (1933) *Arch. Path* 16 330

WINGSTRAND, K. G (1948). *K svenska VetenskAkad.Handl.* III 24 No. 8

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PLEURAL AND HEPATIC AMOEBIASIS TREATED WITH CHLOROQUINE

REPORT OF TWO CASES

BY

NEAL J CONAN, Jr,
JOSEPH A HEAD

AND

ALFRED E BREWER

From the Department of Medicine, New York University College of Medicine, New York

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Chloroquine diphosphate, 7-chloro-4-(4-diethylamino-1-methylbutylamino) quinoline, has produced excellent results in the treatment of hepatic amoebiasis, in the form of acute amoebic hepatitis (CONAN *et al*, 1948, 1949) in the form of hepatic abscesses draining pus containing *Entamoeba histolytica* (MURGATROYD and KENT, 1948, BASNUEVO and GUTIERREZ, 1949, EMMETT, 1949), and in the form of undrained, unaspirated hepatic abscesses (CONAN, to be published) In the last type, one case required subsequent evacuation by needle aspiration of over 3 litres of material containing neither amoebae nor bacteria This, presumably, represented too large a quantity of necrotic debris to be absorbed spontaneously Two other cases of amoebic hepatic abscess (CONAN, to be published), however, resolved without aspiration Yet another successful result (MANSON-BAHR, 1949) has occurred in a case with several previous aspirations and a final one under chloroquine therapy Several of these cases had been unsuccessfully treated with emetine On the other hand, with the same dosage schedule the results of the treatment of intestinal amoebiasis with chloroquine are considerably inferior to those obtained with the usual intestinal amoebacidal drugs (CONAN, 1949) One of these is thus required to supplement the activity of chloroquine in antiamoebic therapy

Another 4-aminoquinoline with high suppressive antiplasmodial activity, sontoquine naphthoate (WISELOGLE, 1946), 3-methyl-7-chloro-4-(-4-diethylamino-1-methylbutylamino) quinoline (nivaquine-M (DECOURT and SCHNEIDER, 1947)), has been studied in human amoebiasis (CONAN, to be published) The naphthoate salt was employed because of a twofold increase in drug concentration within the intestinal lumen when compared with the sulphate salt of sontoquine or the diphosphate salt of chloroquine Despite this perhaps theoretical advantage, sontoquine naphthoate has demonstrated the same qualitative type

of activity as chloroquine viz., excellent in one case of hepatic amoebiasis and *curative in only three out of six cases of intestinal amoebiasis.*

The efficacy of chloroquine against major pleural as well as hepatic manifestations of extraintestinal amoebiasis has now been studied in two instances. They constitute the subject matter of this report.

Case 1 Sharon Hospital \ 908.

A 58-year-old white male railroad section hand, lifelong resident of Dutchess County N.Y., was admitted to the Sharon Hospital, Connecticut, on 12th March, 1949 because of days of severe abdominal pain.

The present illness was of 11 weeks duration, consisting of malaise low grade fever mild cramps and non-bloody diarrhoea. The latter two of these had responded somewhat to symptomatic measures. Six weeks before admission there was a 3- to 4-day period of questionable jaundice and dark urine. This was followed by some further abatement in symptoms, although the low-grade fever persisted. Two days before admission the patient experienced the abrupt onset of sharp agonizing right upper quadrant pain which radiated to the back and rapidly became generalized. Forty-eight hours later he entered the hospital.

On admission the patient was nonicteric, dehydrated, febrile in back and presented board-like abdomen. The temperature was 10?·4 F and the blood pressure 60/50 mm. hg. The white blood cells numbered 40 100 of which 90 per cent. were neutrophils with a marked left-shift. Urinalysis revealed one plus protein and no bile. The serum bilirubin was 0·9 mg per cent. X-ray (Fig. 2) revealed high diaphragm on the right and no ai subdiaphragmatically

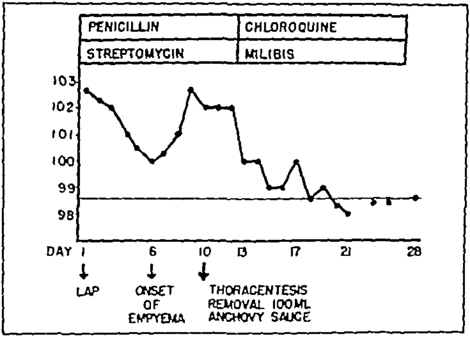

FIG. 1 —Chart showing temperature and other pertinent data of Case 1 Only maximal daily temperatures are plotted here and in Fig. 3.

FIG 2

FIG 3

FIG 2 —Day 1 Prior to laparotomy High right diaphragm
FIG 3 —Day 10 Right empyema prior to thoracentesis

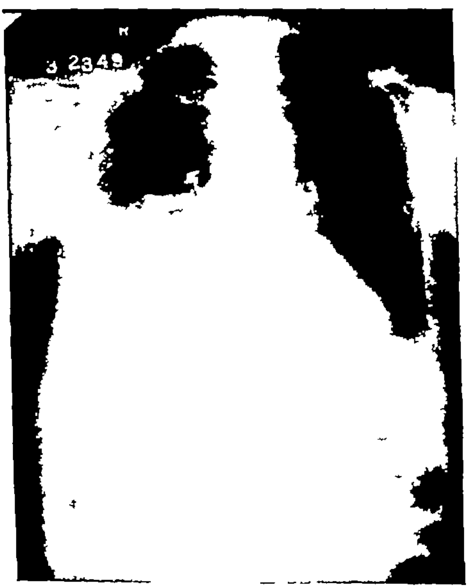

FIG 4

FIG 5

FIG 4 —Day 12 Empyema after thoracentesis Prior to chloroquine
FIG 5 —Day 17 5th day of chloroquine Fluid nearly gone Consolidation seen medially

FIG. 6 FIG. 7

6 — Day 1 9th day of chloroquine \ fluid consolidation less high diaphragm.
Day 28 16th day of chloroquine \ fluid consolidation nearly gone high diaphragm.

Chest X-rays of Case 2

Fig 9

Fig 10

Fig 9—16 III 48 Taken 1 year previously during U R I
Fig 10—23 III 49 9th day of emetine therapy Pleural effusion

Fig 11

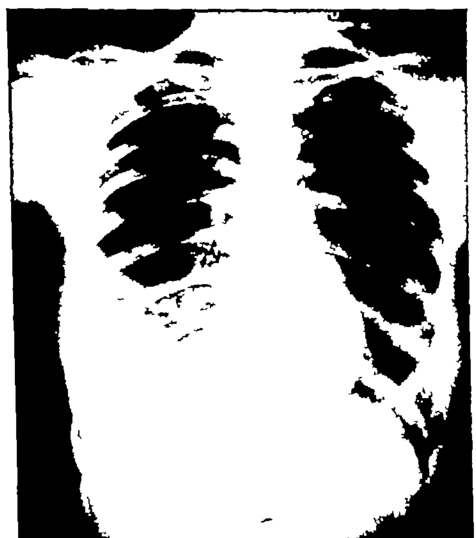

Fig 12

Fig 11—31 III 49 5th day of chloroquine therapy Pleural effusion much less
Fig 12—6 IV 49 11th day of chloroquine therapy Pleural effusion considerably less

Fig. 13

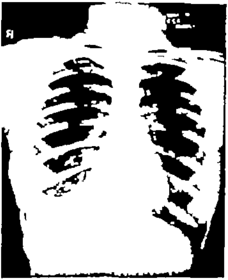

Fig. 14

Fig. 13. 14.IV.4. 11th day of chloroquine therapy. Pleural effusion nearly resolved.
Fig. 14. 25.V.4. 3 weeks after cessation of chloroquine therapy. Pleural effusion gone. High diaphragm and costo-phrenic sinus adhesions.

Following the administration of blood, plasma, electrolytes and water to relieve the shock and dehydration an exploratory laparotomy was performed on the first hospital day The whole peritoneum was found to be covered with creamy pink odourless pus which was discovered to originate from a ruptured abscess cavity (2 × 3 cm) on the superior aspect of the right lobe of the liver Subjacent to this was another abscess cavity (5 × 7 cm) which had not ruptured until this was performed operatively This contained anchovy-sauce pus The two abscesses, having been made to communicate, were drained through a lateral abdominal incision below the costal margin In neither the pink pus nor the anchovy-sauce pus were organisms, protozoal or bacterial, demonstrated in stained smears or by cultures The pink pus was composed primarily of degenerating leucocytes, the anchovy-sauce pus of the same, plus innumerable erythrocytes

Postoperatively, penicillin and streptomycin were administered The drain from the two liver abscesses failed to drain any material and was removed Despite a fall in temperature, the patient's condition deteriorated On the 6th day physical and radiological examination gave evidence of a right pleural effusion This progressed, accompanied by rise in fever on the 8th day (Fig 3) Thoracentesis was performed on the 10th day and about 100 ml of anchovy-sauce pus were removed from the right pleural cavity No subsequent thoracenteses were performed Microscopic and cultural examination of this pus again revealed no organisms, only leucocytes and erythrocytes The patient's condition at this time was nearly terminal Despite the antibiotics, several transfusions, parenteral alimentation, intestinal intubation and suction, there was infection, both localized and spreading, cachexia, and abdominal distention Because anchovy-sauce pus is considered practically pathognomic of infection due to *Entamoeba histolytica*, because frequently the amoebae are not found in the pus itself but rather in the edge of the infected lesion and quite frequently are not demonstrated in material under even open drainage until a day or two following incision, tentative diagnoses of amoebic abscesses of the liver and amoebic empyema were made At this point, however, no faeces were available for examination for amoebae, and purgation in face of the distension and generally critical condition was deemed inadvisable Accordingly, a diagnostic and therapeutic trial was undertaken with a combination of antiamoebic drugs, viz , chloroquine, directed at the extraintestinal infection and milibis* (HAUER, 1943) directed at the intestinal infection The dose of chloroquine was 0 6 gramme of the base by mouth daily for 2 days, followed by 0 3 gramme of the base by mouth daily for 14 days The dose of milibis was two 0 25 gramme tablets thrice daily (1 5 gramme daily) for 16 days The first doses of these were administered at 5 p m on the 12th hospital day Within 11 hours the

* Milibis, para-N-glycolyl arsanilate, is an intestinal amoebacide Supplies of this drug and of aralen (chloroquine) diphosphate and aralen hydrochloride were furnished by Dr A SCRIBNER, of Winthorp-Stearns, Inc

temperature fell from 102 to 99·2° F On the evenings of the 13th, 14th and 17th days the temperature rose as high as 100 F but otherwise remained normal throughout the hospital course Improvement in the patient's general condition was as prompt, as dramatic and as permanent as that of the fever The empyema cleared with gratifying speed (Figs. 4 to 7), and as it did, uncovered a pulmonary consolidation medially which also cleared. The only residue of the infection was an elevation and straightening of the diaphragm. Following discharge the patient returned to work, and presented no new physical findings nor symptoms during the succeeding 6 months.

Case 2. M.H N 65433

A 20-year-old white student nurse was admitted for the third time to the Methodist Hospital, Brooklyn, on 16th February 1949 because of right upper quadrant pain and pain in the right lower chest which radiated to the right shoulder

The only previous illness of any import was acute appendicitis 13 years previously, treated by appendectomy subsequent to which no gastro-intestinal symptoms occurred until the present illness. The family history contributed an item of perhaps epidemiological significance in that one brother had been treated for amoebiasis.

The present illness began in early October 1948 about 4½ months prior to the present admission, with about the same symptom pattern, i.e., malaise anorexia, nausea, mild diarrhoea and pain in the right upper quadrant and in the right lower chest, the latter of which radiated to the right shoulder Physical examination then revealed the liver margin one fingerbreadth below the right costal margin. It was tender both to palpation and percussion. The lungs were negative t physical and radiological examination. The patient was detained in hospital for 6½ weeks, during which she ran intermittent low grade fever (99 to 100 8° F), and experienced no particular progression or regression of symptoms There was never clinical nor chemical evidence of jaundice. Repeated blood counts and urinalyses were within normal limits. Liver function studies revealed persistent mild hypoalbuminaemia and hyperglobulinaemia (each averaging 3·4 gramme per cent.) and moderately positive cephalin-cholesterol flocculation test (+ + to + + + +) The clinical picture was interpreted as persistent low-grade anicteric hepatitis, perhaps viral in origin, and accordingly was treated by dietary methods.

The patient was discharged only to return 2 weeks later for an exacerbation of the same symptoms. At this time physical and laboratory findings were unchanged. Following discharge in December 1948, the patient still had a persistent dull ache in the right upper quadrant and four episodes of more severe pain in this area, accompanied by the right pleuritic pain which radiated to the right shoulder and the malaise anorexia and nausea, but no diarrhoea. This occasioned the present admission in February 1949 At this time the lungs were normal to physical examination and the only positive finding was deep right upper quadrant tenderness to palpation and percussion. Again complete blood counts and urinalyses were within normal limits. The serum proteins, however were now normal as well, although the cephalin-cholesterol flocculation test was + + and the serum alkaline phosphatase activity which had previously been 3 4 Bodansky units per cent. (B.U per cent.) was now 8 7 B.U per cent. As before, there was neither clinical nor chemical evidence of jaundice Fever was now more prominent ranging from 99·6 to 101 4 F Amoebic infection of the liver and pleura was suspected, and although three

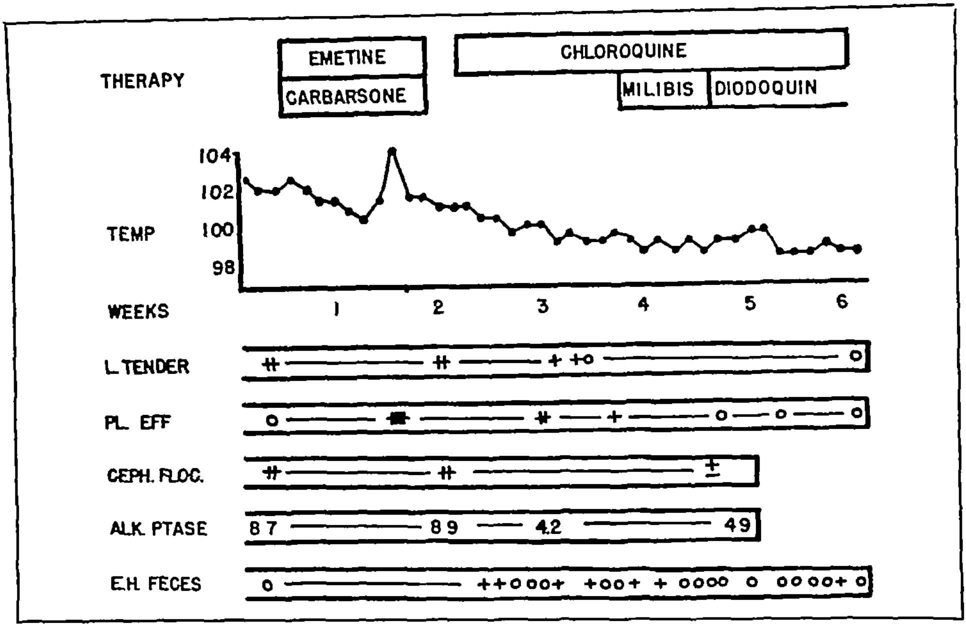

Fig 8 —Chart showing temperature and other pertinent data of Case 2

stool specimens failed to reveal *Entamoeba histolytica,* the serum complement fixation test for amoebiasis was reported positive from the laboratories at the National Institutes of Health Approximately 3 weeks after admission the temperature rose to higher levels (102° F and higher) at which point Fig 8 begins, the first 3 weeks being omitted from it to conserve space Antiamoebic treatment was undertaken over a 10-day period, consisting of emetine hydrochloride intramuscularly in doses of 30 mg twice daily and of carbarsone orally in doses of 250 mg twice daily This treatment was accompanied by mild nausea, diarrhoea and an initial fall in temperature from 102 2 to 100 4° F , but on the 8th day of treatment there occurred a spike in fever to 103 6° F , accompanied by physical and radiological signs of a right pleural effusion (Fig 10) A diagnostic thoracentesis was performed with the removal of only 50 ml of slightly cloudy yellow fluid whose cell count revealed red blood cells and white blood cells in a ratio of three to one No bacteria were demonstrated by stained smears, cultures or guineapig inoculations Upon completion of the course of emetine and carbarsone the patient's symptoms, the hepatic tenderness, the values for serum alkaline phosphatase and the cephalin-cholesterol flocculation test were the same, while there were now two added features, the pleural effusion and the demonstration of both trophozoites and cysts of *Entamoeba histolytica* in the faeces Two days later treatment with chloroquine was instituted in doses of 0 6 gramme of the base daily for 2 days, followed by 0 3 gramme of the base daily for 31 days On the 2nd day of this drug the temperature fell

under 100° F and gradually reached normal values. Within the first week of chloroquine there appeared disturbance in ocular accommodation, frequent nausea and occasional vomiting. These symptoms were not influenced by changing from the oral administration of chloroquine diphosphate to the intramuscular administration of chloroquine hydrochloride (the dose being kept constant in terms of base) over a 4-day period. These symptoms disappeared at the end of the first week concomitant with a considerable reduction in the hepatic tenderness and the pleural effusion (Fig. 11), whose clearing was marked by the appearance of a pleural friction rub. The serum alkaline phosphatase fell from 8·9 B.U. per cent. to 4·2 and, as previously noted, the temperature was now normal. The patient was now asymptomatic except for occasional pain associated with the pleural friction rub which gradually decreased and disappeared in the course of a month. The residual pleural effusion also gradually cleared (Figs. 12 to 14) with there remaining evidence of adhesions in the right costophrenic sinus. The hepatic tenderness was absent by the 10th day of therapy and the cephalin-cholesterol flocculation test when next performed was ±. This much having been accomplished under the influence of chloroquine alone, additional treatment was instituted to help to eradicate the amoebic infection within the colon, which although now asymptomatic was manifest by the continued presence of trophozoites and cysts of *Entamoeba histolytica* in the faeces. After the failure of milibis in doses of 0·250 gramme three times daily for 7 days, diodoquin was administered, followed by disappearance of amoebae from the faeces.

Discussion

The reasoning behind the original testing of chloroquine against hepatic amoebiasis was twofold. (1) To determine whether its antiprotozoal activity as demonstrated against plasmodia might include antiamoebic properties and (2) to test the hypothesis that the differential tissue localization of this drug might possess pharmacological significance. The good results in hepatic amoebiasis as opposed to the poor results in intestinal amoebiasis parallel the tissue localization of this drug. Also conforming with such an hypothesis are the data from parenterally administered emetine which on the one hand indicate greater efficacy against hepatic than intestinal amoebiasis, and on the other hand demonstrate according to the studies of PARMER and COTTRILL (1949) and GIMBLE *et al.* (1948), the same qualitative tissue localization as that of chloroquine.

Furthermore, it would appear that the degrees of hepatic localization of chloroquine previously discussed (CONAN 1949) which are based upon animal work (BERLINER *et al.* 1948) actually obtain in the human, and are perhaps even greater. Nearly simultaneous specimens of plasma and hepatic tissue were obtained at laparotomy from a human volunteer with a normal liver. In order to sample the equilibrium plasma and hepatic concentrations of chloro-

quine following the loading technique of administration, and at a time by which the clinical effect of this drug in hepatic amoebiasis has regularly been demonstrated, the procedure was performed 12 hours following the last dose of 0 3 gramme of the base given once daily for 2 days which were preceded by doses of 0 6 gramme of the base given once daily on the first 2 days The concentrations found* were 0 180 mg per litre in plasma, and 300 0 mg per kg in the liver, a differential of slightly over 1,500 times Since this plasma concentration is nearly identical with the figure of 0 176 mg per litre found as the average equilibrium plasma concentration in a group of humans on maintenance doses of 0 3 gramme of the base of chloroquine (BERLINER *et al* , 1948), these figures may be taken as representative

It is perhaps significant, then, that the hepatic concentration of 300 mg per kg does encompass the range of the *in vitro* amoebacidal concentrations of chloroquine (1-3,500 to + 35,000) obtained in different media and against different strains (CONAN, 1949 , THOMPSON and LILLIGREN, 1949) By the same token, the concentrations in plasma and even more so those in the extracellular fluid (because of the 55 per cent protein binding of the drug) (BERLINER *et al* , 1948), should have no significance, whereas concentrations achieved in either the intestinal wall or lumen would be intermediate, and hence occasionally significant and occasionally not

The pleural cavity and lung are, next to the liver, the commonest sites of extraintestinal amoebiasis If the above hypothesis is correct, chloroquine should be active in pulmonary amoebic infections because it is localized in this tissue to the same extent as it is in the liver While it is true that its concentration in pleural transudate would be lower than in the plasma, in pleural exudate, on the other hand, its concentration would be immensely increased by virtue of its high concentration in leucocytes which, as measured in blood, is 200 times the plasma concentration, and in purulent fluid would be even greater because of the greater number of leucocytes per ml in such fluid as compared to blood

Chloroquine has no serious toxicity of any sort, and its minor toxic effects in the doses used in antiamoebic therapy in over 60 cases observed by the senior author has consisted in five instances of nausea, one of vomiting, two of disturbed ocular accommodation, and one of pruritus All of these were transient and subsided during continued administration In respect to toxicity then it is superior to both emetine and conessine

SUMMARY

Two patients with hepatic amoebiasis developed pleurisy with considerabl effusion, one of which consisted of anchovy-sauce pus and the other of clou

* Dr BERNARD B BRODIE, Research Service, Third (N Y U) Medical Divis Goldwater Memorial Hospital, New York, kindly performed the chemical measurem of chloroquine in this study

yellow fluid. In each instance only a single diagnostic thoracentesis was performed with the removal of 100 ml. or less of fluid. In both cases, subsequent to the administration of chloroquine along with intestinal amoebacidal drugs, there occurred prompt and definite clearing of the effusion, and the inflammation in each anatomical location. In the first case therapy was apparently life-saving The results in these cases are comparable to those reported for emetine (GIMBLE *et al* 1948) and conessine (BERLINER *et al.* 1948 THOMPSON and LILLIGREN 1949). It is emphasized that no therapeutic aspiration of pleural fluid was performed in either instance.

The theoretical considerations arising from these results are discussed.

CONCLUSION

Chloroquine appears as highly effective against amoebic infection of the pleura as it is against amoebic infection of the liver

REFERENCES.

ALAIN M MABRAL, E. TOUZIN R. & PORTE, L. (1949). *Méd. Trop* 9 8.
BARNUEVO, J G & GUTIERREZ, ESTABLE E. (1949). *Rev Kuba Med trop Parasit.*, 5 31
BERLINER, R. W EARLE, D P J TAGGART J V ZUBROD C. G WELCH, W J CONAN, N J J BAUMAN E., SCUDDER, S T & SHANNON J A. (1948). *J clin. Invest.*, 27 98.
CONAN N J J (1948) *Amer J trop Med.*, 28 107
—— (1949). *Amer J Med.*, 6 309
—— (To be published), The Treatment of Amebic Abscess of the Liver with Chloroquine.
—— (To be published, Sontoquine Naphthoate in Amebiasis.
DECOURT P & SCHNEIDER, J (1947) *Bull. Soc Path. exot.*, 40 140.
EMMETT J (1949). *J Amer med. Ass.*, 141 22.
GIMBLE, A. L, DAVISON C. & SMITH, P K. (1948) *J Pharmacol* 94 431
HAUER, A. (1943). *Dtsch. Trop Z* 47 153 (Abstracted in *Trop Dis. Bull.*, 40 913)
MANSON BAHR, P (1949) *J trop Med. Hyg* 52, 91
MURGATROYD F & KENT R. P (1948). *Trans. R. Soc trop Med. Hyg* 42 15
OCHSNER, A. & DEBAKEY M. (1936). *J thorac Surg* 5 225
PARMER, L. G & COTTRELL, C. W (1949). *J Lab clin. Med.*, 34 818.
SOCLAGE, J (1949). *Méd. Trop.*, 1 39
THOMPSON P E. & LILLIGREN B. L. (1949). *Amer J trop. Med.*, 29 323
WISELOGLE, F Y (1946). *A Survey of Antimalarial Drugs, 1941 1945* 1 382, Ann Arbor J W Edwards.

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ONYALAI IN THE BECHUANALAND PROTECTORATE *

BY

BERNARD T SQUIRES

The occurrence of onyalai, a form of thrombocytopenic purpura, has, of late years, been reported from many different parts of Africa, ranging from Kenya to the Cape Province of the Union of South Africa

The disorder was first noted in the Bechuanaland Protectorate by MORGAN and SQUIRES (1940), who reported 24 cases, a further series of 52 cases was published by SQUIRES (1943) This paper records another series of 35 cases, which were admitted to hospital, and 71 mild or abortive cases seen either at out-patient clinics or in the course of routine inspections

Clinical Features

These have been described in detail by BLACKIE (1937) and GEAR (1938), a brief recapitulation only will be given Two stages in the progress of the disease can be recognized, the prodromal and the haemorrhagic The prodromal stage is characterized by headache, malaise, and generalized pain Occasionally the tongue becomes swollen, and the patient may complain of transient numbness in various regions, there is often a pyrexia, up to 102° F

The duration of this stage, so far as can be ascertained, varies from 1 to 3 days

* I am indebted to the Acting Director of Medical Services of this territory for permission to record these notes

The haemorrhagic stage is ushered in by the oozing of blood from the mucosa of the mouth and nose, and by the appearance of haemorrhagic bullae in the mucous membranes and skin. These bullae are tough, trabeculated sometimes umbilicated, and contain clot they vary in size from that of a pin's head to a mass 1 inch in diameter

In a moderately severe typical case the number of visible bullae increases for the first 3 or 4 days, and then begins to diminish. An example (Table 1) is given from such a case which exhibited only one bulla at the time of admission to hospital.

TABLE 1.

Day	Number of bullae in mouth.	Day	Number of bullae in mouth.
1	1	7	8
2	7	8	6
3	9	9	3
4	12	10	1
5	10	11	1
6	8	12	—

In severe cases, haemorrhage occurs from the gastro-intestinal and genito-urinary tracts, so that haematemesis, melaena, haematuria, and vaginal bleeding are encountered. In such cases, the bleeding may be so severe and extensive that the patient dies within 2 or 3 days. BLACKIE (supra) and GEAR (supra) mention the occurrence of cerebral haemorrhage, but no such involvement has been observed in cases seen in this territory

Laboratory Findings.

Two constant features are found in blood examinations. The first is a remarkable diminution almost to vanishing point in the number of platelets. BLACKIE (supra) states that the platelet count may be as low as $\pm$ 1 000 the lowest count found by the writer was $\pm$ 5 000 and the highest $\pm$ 20,000

The second feature is the prolonged bleeding time the bleeding time found in healthy African f this territory is 2 to 6 minutes, but n cases of onyalai bleeding times up to 90 minutes have been encountered. In contrast to the bleeding time the coagulation time is within normal limits Otherwise the blood picture in general is that characteristic of an acute normocytic anaemia following extensive haemorrhage

Morbid Anatomy

Postmortem examination demonstrates the presence of haemorrhagic effusion into most of the serous cavities. The most striking appearances are

found in the gastro-intestinal and genito-urinary tracts The gut may be full of haemorrhagic areas and bullae, from stomach to rectum , such areas are also found in the renal pelves, bladder, and urethra

Mortality

This varies greatly in different series, as shown in Table II

TABLE II

Author	Number in series	Deaths	Mortality, per cent.
Blackie, 1937	7	1	14
Gear, 1938	7	3	43
Gilkes, 1934	53	15	29
Gilkes, 1934	17	8	47
Morgan & Squires, 1940	24	—	—
Squires, 1943	52	5	9

DIFFERENTIAL DIAGNOSIS

Onyalai is differentiated from other forms of purpura by the pathognomonic bullae If no bullae are visible, onyalai can be distinguished from Schonlein's purpura by the absence of joint pains and of initial sore throat, and from Henoch's purpura by the absence of colic, and from both by the severe deficiency in platelets, which seldom occurs in these two diseases Werlhof's disease seems to bear a great similarity to onyalai, but the bullae characteristic of onyalai have not been described in Werlhof's disease

TREATMENT

A variety of treatments have been described , the sheet anchor of most of them is the exhibition of some form of styptic or haemostatic, such as calcium or iron in sundry forms , but it is doubtful as to whether these are really of any use Blood transfusion has been employed with success, and also intramuscular injection of whole blood, 20 ml at a time In rural areas, the latter method is far simpler, and appears to be as good as transfusion in its results

PROGNOSIS

Mild cases recover spontaneously In regard to severe cases, the writer's experience is that however grave they may appear, so long as there is no macroscopic haematuria, they will recover , conversely, if an apparently mild case develops haematuria, the prognosis becomes correspondingly serious

PRESENT SERIES.

The salient points of interest of this series of 35 cases are given below

Sex Distribution.—Males, 22 females, 13. The preponderance of males was noted in other series (MORGAN and SQUIRES SQUIRES, supra).

Age Distribution.—This is given in Table III The range was 1 to 60 years.

TABLE III.

Age group, years.	Number	Age group years.	Number
1 10	11	21–30	8
11 20	12	Over 30	4

It will be noted that two-thirds of the patients were under 11

Number of Days in Hospital.—Mean 11 Range 2 to 38.

Deaths.—There were three deaths in the series giving a mortality of 8 per cent. All deaths occurred in the youngest age group. Two patients had a familial history and three had suffered previous attacks.

The Mild or Atypical Case

(2) An African female, aged c. 25, complained of headache and malaise for 2 days. She stated that vaginal bleeding had begun that morning, although her next period was not due for a week. She was apyrexial and routine examination was negative. A vaginal examination, however, revealed the presence of a solitary bulla behind the carunculae myrtiformes, with some haemorrhage oozing. She refused haemotherapy but was persuaded to remain in the neighbourhood for a few days and given a placebo. Next day two bullae were present in the mouth.

No more appeared and 2 days later all three bullae had cleared up. At this time the patient stated that she felt perfectly well.

Routine examination of African school children has also revealed cases so mild that the patient has not even stayed away from school. Such cases can often be detected after spontaneous cure if the bullae have occurred in visible sites; these exhibit a small reddened, scarred area which persists for a day or two. A large number of these cases masquerade as cases of epistaxis. The following case is typical.

(3) An African school boy, aged 10, was seen at a routine examination of school children. The teacher stated that on the day before the child had had an epistaxis, with headache and lassitude. Inspection revealed a number of tiny black spots about 1 mm in diameter, on the buccal mucosa. On magnification these were seen to be typical small bullae, some of which were oozing blood. Two days later they had disappeared.

COMMENT

The aetiology of onyalai is still obscure. There is as yet no evidence of the existence of any infective agent, and it is the rule for cases to occur singly, even where there is a familial history. Further, if the disease is of infective origin, one attack does not appear to confer lasting immunity, for cases of repeated attacks have been recorded.

It has been suggested that onyalai is a manifestation of poisoning, due to toxic agents such as might be contained in native medicine.

Since it is practically impossible to confirm that any such medicine has or has not been taken before the patient comes to hospital or dispensary, the theory is equally hard to prove or disprove. Many of the writer's patients have emphatically denied that they had taken native medicine before coming for treatment. Further, the cases seen in school children who had not even stayed away from school are evidence against the correctness of this theory.

There is no correlation between the incidence of onyalai and that of malnutrition; indeed, most cases occur in well-nourished individuals.

No seasonal or topographical influence on the incidence of the disease can be traced, for it occurs at all times of the year, and with like frequency amongst the Tswana, who live in well-watered agricultural areas, and the inhabitants of the Kalahari desert.

At present, therefore, the aetiology is obscure, and onyalai must be regarded as an idiopathic thrombocytopenia, with a unique lesion, the haemorrhagic bulla.

Summary of Contents.

1 Clinical, postmortem, and laboratory findings characteristic of onyalai are described, together with treatment and prognosis.

2 A series of 35 cases in patients admitted to hospital is recorded, and the salient points connected therewith.

3 The frequent occurrence of mild or atypical onyalai is emphasized, with three typical case histories, selected from 71 cases.

4 The aetiology is briefly discussed.

REFERENCES.

Blackie, W. K. (1937). *Trans R. Soc trop. Med Hyg* 31 207
Gear, J (1938). *S Afr med. J.*, 12 632.
Gilkes, H A. (1934). *Trans. R. Soc. trop Med. Hyg.*, 27 491
Morgan A. A. & Squires, B. T (1940) *S Afr med. J* 14 231
Squires, B. T (1943) *Ibid.*, 17 282.
Stein H. B. & Miller, E. (1943). *S Af J med. Sci.*, 8 1

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SEASONAL VARIATIONS IN RAINFALL PREVALENCE OF *HAEMAGOGUS* AND INCIDENCE OF JUNGLE YELLOW FEVER IN BRAZIL AND COLOMBIA *

BY

HENRY W KUMM

The Rockefeller Foundation

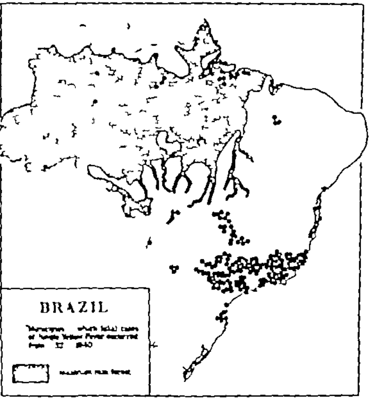
Yellow fever without *Aëdes aegypti* was reported for the first time by SOPER *et al* (1933) in a rural area in the Vale do Canaã, State of Espírito Santo, Brazil It was not until several years later, however, that the tremendous extent of the affected area in South America and the importance of the problem itself came to be fully realized The term "jungle yellow fever" was coined by SOPER (1936) to differentiate the sylvan variety of the disease from rural yellow fever transmitted by *Aëdes aegypti*

Since the initial epidemic in the Vale do Canaã a total of 1,224 fatal cases of jungle yellow fever have been diagnosed in Brazil The region involved is indicated on Maps 1 and 2, and may be subdivided phytogeographically I

* The studies and observations on which this paper is based were conducted with th support and under the auspices of the Yellow Fever Research Service, which is main tained jointly by the Ministry of Education and Health of Brazil and by the Internation Health Division of The Rockefeller Foundation

It is a pleasure to acknowledge the help received from many collaborators in th preparation of this report Dr AUGUSTO GAST-GALVIS, of Bogotá, assembled the da on the incidence of fatal human cases of jungle yellow fever in Colombia during the peri from 1934 to 1947 Information concerning the areas of Brazil covered by the Amazon type of rain forest was obtained from Dr G M DE OLIVEIRA CASTRO and Dr HENRIQ P VELOSO, of the Oswaldo Cruz Institute, as well as from airplane pilots of the Pan do Brasil, the Cruzeiro do Sul and the Brazilian Air Force

the area covered by the Amazonian type of rain forest a few cases of yellow fever have been encountered each year since 1932, and at all times during the calendar year although owing to inaccessibility probably only a small proportion of the actual cases have been officially notified. In the remainder of Brazil, on the other hand particularly those areas from which records are more reliable, two epidemics involving the months from November to June are known to have



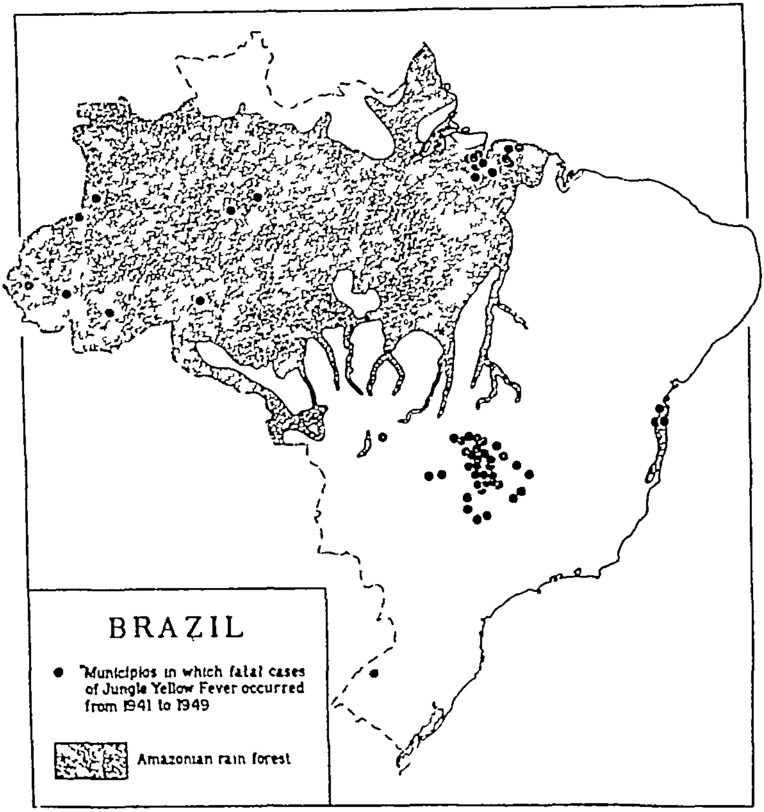
Map 1. The geographical distribution of fatal human cases of jungle yellow fever in Brazil from 1932 to 1940 as related to the area covered by the Amazonian type of rain forest

occurred, the first extending from 1934 to 1940 and the second from 1944 to 1945. There is a striking duplication in some of the areas invaded in the 'thirties as compared to those attacked during the forties.

Meanwhile in Colombia 468 liver specimens positive for yellow fever were encountered during the 14-year period extending from 1934 to 1947. The two principal endemic areas of that republic are separated by the eastern Cordillera of the Andes, but nevertheless the seasonal incidence of the disease is

similar in both of them showing a bimodal distribution with peaks in July and December

A striking difference between the sparsely and the heavily forested regions of Brazil is brought out by a study of the seasonal incidence of jungle yellow fever in those two areas In the zone not included in the Hiléia Amazônica cases usually begin to appear in November or December and reach a peak in



MAP 2 The geographical distribution of fatal human cases of jungle yellow fever in Brazil from 1941 to 1949 inclusive, as related to the area covered by the Amazonian type of rain forest

February, while there are no recorded fatalities during the 4-month period from July to October inclusive In the Amazonian rain forest and the cocoa-growing areas near Ilhéus, on the other hand, the incidence curve is bimodal with high points in January and again in July Climatic conditions in the affected areas of Colombia are similar to those prevailing in the Hileia Amazônica

It should not be inferred from Maps 1 and 2 that the Amazonian rain forest is the only large sylvan area remaining in Brazil Tropical rain forests of considerable extent still flourish near the sea coast of several of the southern

states, though towards the interior these wooded areas are being cut back severely and in some regions have been almost completely destroyed.

Data on rainfall, in most cases covering periods of more than 10 years, are available from 20 localities situated in the Hiléia Amazônica. These include two places in the Acre Territory and one in Guaporé as well as nine in the State of Amazonas and eight in Pará. The number of years in each locality during which recent rainfall records have been carefully kept are indicated in Table I. In addition, similar figures have been obtained from three places situated in the cocoa-growing region near Ilhéus in southern Bahia. In Table III the monthly rainfall figures for the Ilhéus area have been combined with those from the Amazon river valley in calculating the average rainfall per month for the entire area of Brazil which is covered by the Amazonian type of rain forest.

In the remainder of Brazil records from two localities in Mato Grosso, two in Goiaz, one in São Paulo and four in Minas Gerais, which are listed in Table I, have been combined in order to calculate the monthly averages that are quoted in Table II.

Similarly in Colombia, reliable records have been secured from three places in the valley of the Magdalena river as well as from Villavicencio, which lies at the foot of the eastern range of the Andes. These figures have been added together and the monthly averages computed to provide an indication of conditions existing in the areas of Colombia where jungle yellow fever prevails.

In the regions of Brazil other than the Amazon river valley and the Ilhéus area, which are for the most part sparsely forested, it is possible to compare rainfall and *Haemagogus* prevalence with yellow fever incidence. Average monthly rainfall figures were calculated from the data indicated in Table I and the 1 138 fatal cases of jungle yellow fever diagnosed by the viscerotomy service in this zone have been arranged by date of onset. At Passos, in southern Minas Gerais, CAUSEY and DOS SANTOS (1949) have carried out systematic daytime captures of *Haemagogus* and other forest mosquitoes for more than 2 years. The percentages of the total number of *Haemagogus* caught, which were taken by them each month, have been calculated for comparison with rainfall and yellow fever incidence. Table II and the lower portion of the accompanying graph show that the peak of rainfall occurs in December, the greatest prevalence of *Haemagogus* in January and the maximum incidence of yellow fever cases in February.

Because the regions of Brazil other than the Amazon river valley and the Ilheus area lie well south of the Equator, the year in Table II, as well as that portion of the graph referring to the sparsely forested areas, begins in July instead of in January.

The graph also shows that in the heavily forested zones both of Colombia and of Brazil, bimodal curves of yellow fever incidence are encountered, with cases confirmed in every month of the year. The percentage of *Haemagogus*

mosquitoes caught each month in Colombia has been taken from the article by GAST-GALVIS and BATES (1945) The Colombian species designated as *Haemagogus capricornii* by GAST-GALVIS and BATES (1945) was shown later by KUMM, OSORNO-MESA and BOSHELL-MANRIQUE (1946) to be a variety of *Haemagogus spegazzinii*

TABLE I —PLACES IN BRAZIL AND COLOMBIA FROM WHICH RAINFALL RECORDS HAVE BEEN OBTAINED

State, territory, department or intendencia.	Locality	Number of years for which rainfall records are available
BRAZIL		
Acre Territory	Cruzeiro do Sul	14
	Sena Madureira	26
State of Amazonas	Boca do Acre	12
	Caruarí	11
	Coari	18
	Fonte Boa	14
	Javarete	12
	São Gabriel do Rio Negro	20
	São Paulo de Olivença	12
	Taracua	19
	Tefé	12
State of Bahia	Água Preta	6
	Belmonte	11
	Ilhéus	27
State of Goiaz	Catalão	31
	Goiaz Velho	22
Guaporé Territory	Porto Velho	11
State of Mato Grosso	Campo Grande	11
	Três Lagoas	14
State of Minas Gerais	Juiz de Fora	14
	Lavras	14
	Paracatú	14
	Uberaba	14
State of Pará	Belém	19
	Clevelândia	19
	Obidos	14
	Porto Moz	14
	Salinas	15
	Soure	13
	Taperinha	28
	Tomé Açú	5
State of São Paulo	São Carlos	14
COLOMBIA		
Department of Santander	Barranca Bermeja	22
	El Centro	10
Department of Tolima	Honda	6
Intendencia of Meta	Villavicencio	13

In Colombia the first peak of rainfall occurs in May and is followed by a high point of *Haemagogus* prevalence in June, and a month later by the first peak of yellow fever cases. There is however no pronounced secondary rise in the abundance of *Haemagogus*. For that reason GAST-GALVIS and BATES (1945) suggested that the second peak of yellow fever incidence in Colombia was brought about by certain local customs of the people which involve cutting down portions of forest in November and December to enable them to burn the trees during the dry months and to have fresh land available for planting

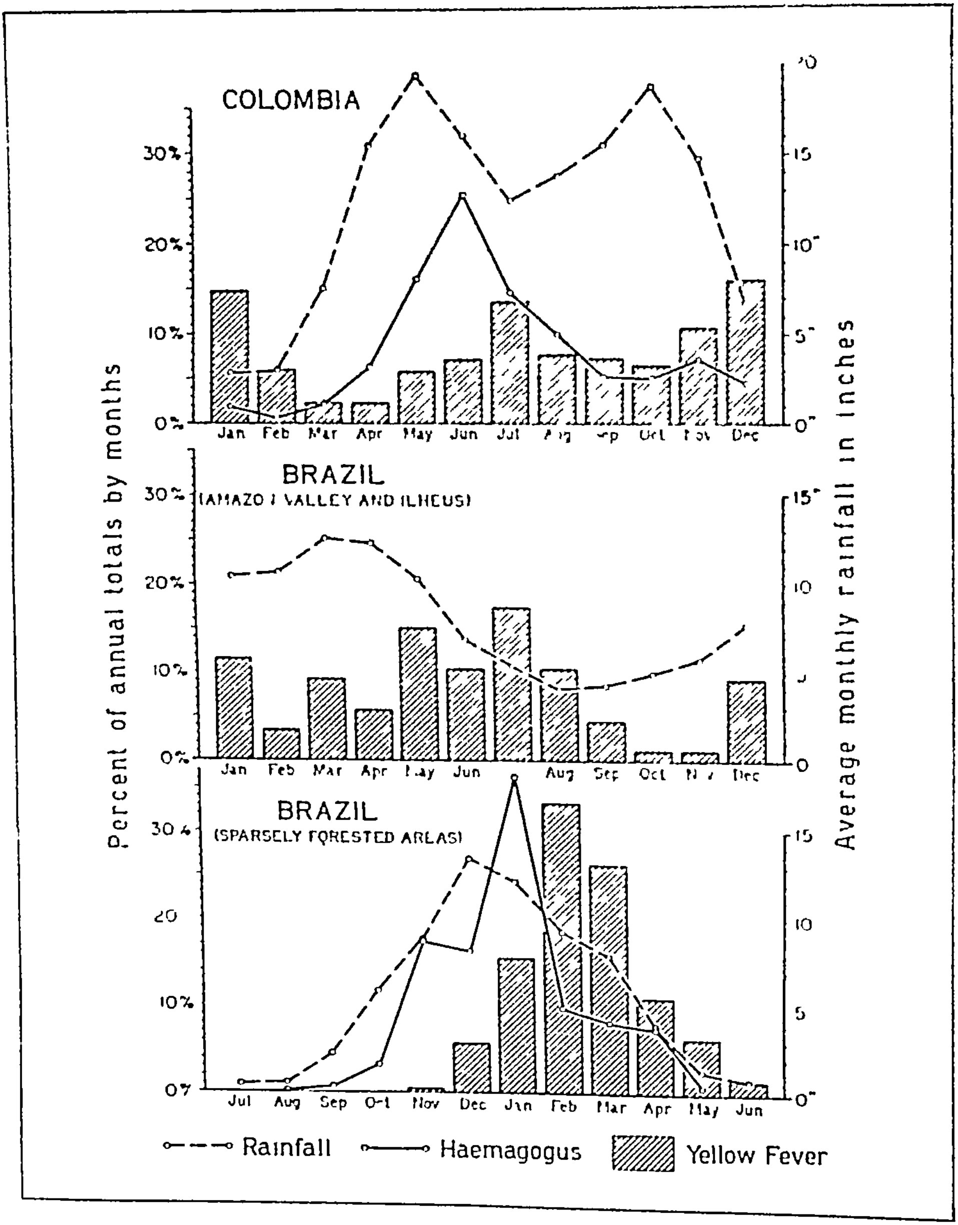
TABLE II.—SEASONAL VARIATIONS IN RAINFALL, PREVALENCE OF *Haemagogus* AND INCIDENCE OF JUNGLE YELLOW FEVER IN REGIONS OF BRAZIL OTHER THAN THE AMAZON VALLEY AND THE ILHÉUS AREA.

Months.	Rainfall in inches.	Adult *Haemagogus*.		Cases of yellow fever	
		Number caught.	Per cent. of total each month.	Number diagnosed.	Per cent. of total each month.
July	0·43	2		—	—
Aug	0·52	4	0·1	—	—
Sept.	2·23	57	0·6	—	—
Oct.	5·83	[illegible]	3	—	—
Nov	8·85	1,643	17·4	4	0·4
Dec.	13·43	1,406	16·3	85	8·7
Jan.	12·01	2,263	26·2	178	16·4
Feb.	9·24	877	9·6	278	23·2
Mar	7·63	720	8·1	294	20·2
Apr	3·90	654	7·4	128	11·1
May	1·31	70	0·8	73	6·4
June	0·77	4	—	18	1·6
Total	66·51	8,096	100·0	1,139	100·0

Fatal cases of jungle yellow fever diagnosed by the viscerotomy service and classified by date of onset of disease.

at the onset of the rains. Thus, a portion of the human population in Colombia would come into closer contact with the forest in November and December than at any other time of the year

In the Amazon valley and in the vicinity of Ilhéus, the maximum rainfall occurs from January to May with a crest in March followed 2 months later by the first peak of human yellow fever fatalities. It is likely that the higher incidence of sylvan yellow fever in the months from May to August inclusive may be explained by the habits of the people as well as by fluctuations in rainfall. In the State of Pará for example, the important crop of Brazil nuts is usually gathered between May and September Frequently the clusters of nuts are picked up from the ground, but if there is any great urgency men may climb



GRAPH

Seasonal variations in rainfall, prevalence of *Haemagogus* and incidence of jungle yellow fever in Colombia, the heavily forested Amazon valley and Ilhéus regions, and the sparsely forested areas of Brazil

rtholletia trees to speed up the collection of the nut harvest Such s would be working in the forest canopy in the very zone where *Haema-* mosquitoes are most abundant An explanation of the secondary rise s of jungle yellow fever in Brazil during December and January is not nt, although at Ilhéus there was a slight increase in the monthly rainfall the month of November

III —SEASONAL VARIATIONS IN RAINFALL, PREVALENCE OF *Haemagogus* AND INCIDENCE OF YELLOW FEVER IN COLOMBIA COMPARED TO RAINFALL AND YELLOW FEVER IN THE AMAZON VALLEY AND THE ILHÉUS AREA OF BRAZIL

nths	COLOMBIA East and west of the eastern cordillera of the Andes				BRAZIL Amazon river valley and the vicinity of Ilhéus		
	Rainfall in inches	Per cent of adult *Haemagogus* caught each month	Cases of yellow fever * Number diagnosed	Cases of yellow fever * Per cent of total each month	Rainfall in inches	Cases of yellow fever * Number diagnosed	Cases of yellow fever * Per cent of total each month
.	2 82	1 9	69	14 7	10 41	10	11 6
)	2 96	0 6	28	6 0	10 68	3	3 5
r	7 58	2 1	11	2 3	12 55	8	9 3
r	15 49	6 4	11	2 3	12 28	5	5 8
y	19 31	16 2	27	5 8	10 29	13	15 1
ne	16 04	25 5	33	7 1	6 86	9	10 5
y	12 42	14 7	63	13 5	5 40	15	17 4
g	13 78	10 1	36	7 7	4 07	9	10 5
ot	15 50	5 4	34	7 3	4 28	4	4 6
t	18 73	5 2	31	6 6	4 95	1	1 2
ov	14 73	7 3	50	10 7	5 74	1	1 2
c	6 71	4 6	75	16 0	7 70	8	9 3
tals	146 07	100 0	468	100 0	95 21	86	100 0

Fatal cases of jungle yellow fever diagnosed by the viscerotomy service and classified by onset of disease

BATES (1945) pointed out that in the Cuchilla ravine near Villavicencio, nbia, enough *Haemagogus* were present at all times of the year to maintain mic jungle yellow fever In the sparsely forested areas of Brazil, however, itions are very different, since there is a period of at least 4 months during h rainfall almost ceases, atmospheric temperature falls, *Haemagogus* catches me insignificant, and jungle yellow fever itself does not occur

At first one is tempted to conclude that the Amazon valley and Ilhéus areas of Brazil, as well as similar regions in Colombia, belong to an extensive endemic zone of jungle yellow fever in South America. In the remainder of Brazil at least two known human epidemics have appeared separated by an interval of 10 years. It would be incorrect to state, however that any particular areas in one region are permanently endemic while those in the other are subject to periodic epidemics, because there is no known locality even in the Hiléia Amazônica itself where jungle yellow fever has been constantly present for many years. Instead, the entire region is characterized by wandering epizootics among the primates and by occasional human cases as well. The outstanding difference between the areas of Brazil which are covered by the Amazonian type of rain forest and the rest of the country is that in places situated in the former human outbreaks of jungle yellow fever recur more frequently than they do in the latter

Summary

The seasonal distribution of rainfall, *Haemagogus* mosquitoes and jungle yellow fever in the heavily forested areas of Brazil is similar to that in Colombia but different from that encountered in the remainder of Brazil. In the sparsely forested zone of Brazil climatic conditions from July to October are unfavourable for the disease and for its sylvan vectors.

REFERENCES.

Bates, M. (1945). *J Anim Ecol.*, 14 17
Causey O R. & Dos Santos, G V *Amer J Hyg* (In press.)
Gast-Galvis, A. & Bates, M. (1945). *Rev Fac. Med. Bogotá*, 14 43
Kumm, H. W Osorno-Mesa, E. & Boshell-Manrique, J (1946) *Amer J Hyg* 43 13.
Soper, F L. (1936). *Rev Hyg Saude publ.*, 10 107
——, Penna, H., Cardoso E. Serafim J J Frobisher, M., J & Pinheiro, J (1933). *Amer J Hyg* 18, 555.

TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE
Vol 43 No 6 May, 1950

A SHORT ACCOUNT OF THE TYPES OF DYSENTERY IN SIERRA LEONE

WITH REPORT OF A CASE OF INFECTION WITH *SHIGELLA SHIGAE* *

BY

JOHN D REID,
Pathologist, Colonial Medical Service,

AND

MINNIE GOSDEN,
Senior Pathologist, Colonial Medical Service
From the Government Laboratory, Sierra Leone

Shiga dysentery is rare on the West Coast of Africa The isolation of *Shigella shigae* in July, 1949, at Freetown is thought to be worth recording, and also prompts us to review the incidence of the different types of dysentery bacilli isolated at the Government Laboratory, Freetown

This laboratory serves the Connaught Hospital with 240 beds, the Maternity Hospital with 40 beds, the Mental Hospital with 180 patients, the various out-patient clinics, and the Health Department An average of 230 specimens of faeces are examined per month For routine examination, fresh saline and iodine preparations, and a simple concentration in saturated salt are used All specimens suggestive of dysentery are cultivated by plating on desoxycholate citrate agar or McConkey agar

* Our acknowledgements are due to Dr F MACLAGAN, Director of Medical Services, for permission to publish this paper, and to Mr C W STONE, Laboratory Superintendent, for his technical assistance

It is difficult to assess the relative incidence of amoebic and bacillary dysentery. During the 36 months from October 1946 to September 1949, the period covered by this review, 95 cases of amoebic dysentery and 123 cases of bacillary dysentery were found. Amoebiasis was probably commoner than these figures suggest, as a few patients came to autopsy without the diagnosis having been made, and some were brought in dead. Between 1947 and 1948 amoebiasis accounted for 36 deaths out of 1,202 autopsies. An occasional case was found in which the colon was riddled with ulcers, yet the faecal contents were apparently normal and no pus cells were reported in the specimen received before death.

Seventeen cases of bilharzia dysentery were found in the 36 months under review. This condition is more prevalent in the Protectorate.

All the common types of dysentery bacilli except *Sh. boydii* were found. As we have been using *Sh. boydii* serum only during the past 12 months, it is possible that some of the untyped "Flexner" group bacilli may have belonged to this type.

The following is the actual incidence of the organisms isolated.

TABLE I.

Sh. flexneri.							*Sh. sonnei.*	*Sh. schmitz*	*Sh. shigae*	*Bacterium alkalescens.*
Type.	V	W	Z	103	119	Newcastle.				
20	6	33	19	8	15	5	10	4	1	1

Sh. flexneri W is the commonest type. It is also probably the most virulent, as the only fatal case of acute bacillary dysentery which occurred during this period was due to this organism. The patient was a well nourished African man of about 25 years old, and died within 4 days. At autopsy the whole colon was intensely inflamed; the para-aortic and mesenteric glands were enlarged; there were petechial haemorrhages in the heart and stomach; the liver and kidneys showed fatty changes and the spleen was enlarged and soft. *Sh. flexneri* W was isolated from the faeces before death and also from the colon at autopsy.

All the bacillary types have recurred each year with the exception of *B. alkalescens* and *Sh. shigae*.

Since the reconstitution of this laboratory in 1940 only one case of shiga dysentery has been found. This occurred in July 1949. The patient was a young African man who had been working on the wharf. Clinically the case was mild and non-toxic, but it took 9 days to clear up instead of the usual 2 or 3 days. *Sh. shigae* was isolated on two occasions. The bacillus was a non-motile rod which fermented glucose in 1 day and maltose in 9 days without the

production of gas It failed to ferment mannite, lactose, saccharose, dulcite, rhamnose, xylose or arabinose It did not produce indole in 7 days It agglutinated *Sh shigae* serum (Standards Laboratory) to titre, and failed to agglutinate with serum absorbed with N C T C 4873 *Sh shigae* The latter failed to agglutinate with serum absorbed with our strain

The following table shows the monthly number of cases of enteritis, including dysentery (*i e*, all specimens with blood, mucus or pus), bacillary dysentery, amoebic dysentery, enteric fever, ascariasis and ancylostomiasis found during the 36 months under review

TABLE II

Total	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct.	Nov	Dec	Total
Specimens faeces	683	634	649	683	615	539	808	789	757	986	739	621	8,503
Enteritis	140	162	123	145	154	149	264	271	213	229	188	170	2,208
Bacillary dysentery	7	—	1	3	12	12	21	33	16	10	5	3	123
Amoebic dysentery	12	10	5	5	11	2	10	15	5	5	8	7	95
Enteric	1	8	11	7	5	2	5	10	11	16	18	25	119
Ascaris ova	119	113	98	82	101	116	109	108	114	109	104	120	1,293
Ancylostome ova	81	109	76	70	73	85	70	74	85	129	101	88	1,041

Bacillary and amoebic dysentery occur throughout the year Bacillary dysentery is most prevalent during the rains of July and August Amoebic dysentery, like ascariasis, shows less seasonal incidence, and is probably spread by direct contact Houseflies are not so prevalent here as they are in many other tropical countries, but it is probable that they play their part in the seasonal incidence of bacillary dysentery If water were the source of infection, one would expect enteric fever to show a similar incidence

SUMMARY

A case of *Sh shigae* dysentery is reported from Freetown, together with an account of the type of dysentery found there All common types of dysentery bacilli have been isolated, except *Sh boydii* Bacillary and amoebic dysentery are equally common, but bacillary is commoner during the rainy season

TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE
Vol 43 No 6 May, 1950

CORRESPONDENCE.

To the Editor

Shigella INFECTION IN *Ursus arctos*

SIR,—We thought you might be interested in a case of naturally acquired dysentery in a brown bear This animal, an *Ursus arctos*, was a present from the Bern Zoological Garden to the Governor General of the Belgian Congo It came by boat via Antwerp and Matadi, and arrived in Léopoldville on the 15th November, 1949 The next day it presented diarrhoea A direct examination of the stools showed no parasites, but culture gave numerous colonies of *Shigella flexneri* Further identification of the strain showed that it was *Sh flexneri* type WX (ANDREWES and INMAN)

The brown bear recovered uneventfully without special treatment, and up to date is perfectly healthy, living in the Zoological Garden at Léopoldville Subsequent stool-cultures were negative We believe it is the first time a Shigella strain has been isolated from a spontaneous infection of such an animal

We are, etc ,

Dr E L VAN OYE,
Laboratoire médical, Léopoldville

Lt -Col R F BRIDGES, M B ,
Dysentery Reference Laboratory, Oxford

16*th February*, 1950

RHEUMATIC FEVER

SIR,—With much interest I read the paper of Dr BARNES on rheumatic fever in Fiji I wish to point out that in 1946, in a paper which I published together with Dr VAN DER SAR,* we reported on the occurrence of rheumatic carditis in the native (Negro) population of Curacao, N W I Among 3,391 admissions for internal diseases over a period of 5 years, there were 61 for acute rheumatic fever Three cases were complicated by chorea minor Among 1,307 autopsies there were 20 which disclosed typical gross lesions or sequelae of rheumatic carditis In 12 of these, histologic examination was possible, and in 11 typical Aschoff bodies were found

As Curacao is lying at about 12° Northern latitude, it must certainly be considered a tropical island, and our findings are certainly a confirmation of the opinion that the non-finding of rheumatic carditis in the population of the tropics is caused by the paucity of postmortem examinations

I am, etc ,

PH H HARTZ,
Pathologist to the Public Health Service, Curacao

27*th March*, 1950

* *Arch Path* (1946), 41, 32

TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE
Vol. 43 No. 6 May 1950

OBITUARY

SIR ARTHUR WILLIAM GARRARD BAGSHAWE, C.M.G., B.A., M.B. (CAMB.).

The Royal Society of Tropical Medicine and Hygiene has been privileged to number many men of world-wide scientific reputation among its Presidents, and these men have counted their election to that distinguished office among the crowning achievements of their careers. In this great company Sir Arthur Bagshawe was at home, by right of character and achievement though he early left the field of research for that of record and the collating of other men's work.

He was born on 29th July 1871 the second son of the Rev Alfred Drake Bagshawe rector of Wormhill, near Buxton. He was educated at Marlborough and went up to Cambridge where he took his Natural Sciences Tripos his medical education was completed in 1895 at St. George's Hospital. His early interest was in botany and it was no doubt with the object of satisfying this interest, as well as of practising in the then still largely unknown field of tropical medicine, that he joined the Uganda medical service in 1900 In East Africa he served on the Lango Expedition in 1901 and on the Anglo-German Boundary Commission in 1902–4. He soon developed an interest in the insects of medical and veterinary importance, particularly the tsetse flies, and he was the first to discover the pupa of *Glossina palpalis* in the natural haunts of the fly

It was in 1908, however that Bagshawe was given the opportunity to develop the bent for which he will best be known, when he was requested to create in London the Sleeping Sickness Bureau, whose object was to collect, from the medical literature of the world, information relating to trypanosomes and their vectors, and the diseases of man and animals to which they give rise, and to collate and publish accounts of this information in a form suitable for use in the field and in the laboratory On a budget which in modern times seems to be fantastically small, Bagshawe did this work, and himself wrote the four volumes of the *Sleeping Sickness Bulletin* which appeared in monthly parts during the years 1908–12. The condensation of such a mass of information called for qualities of judgment in the selection of important material and the rejection of trivial work, and of accuracy in reproduction, which suited Bagshawe's mind,

and he created a standard of medical abstracting which itself could be ranked as a scientific feat The Sleeping Sickness Bureau was soon enlarged to include the production of the *Kala Azar Bulletin* edited under Bagshawe's direction by Dr C M Wenyon, and the *Tropical Disease Bulletin*, which included the others, and which began its career in 1912 The Bureau expanded further, under Bagshawe's direction, to publish the *Tropical Veterinary Bulletin*, the *Sanitation Supplements of the Tropical Diseases Bulletin*, the *Bulletin of Hygiene* and the *Supplement* to the *Tropical Diseases Bulletin* In all these publications he received great assistance from Fellows of the Royal Society of Tropical Medicine and Hygiene

Bagshawe's active services to the Royal Society of Tropical Medicine and Hygiene were made during a continuous period of 26 years He was a member of Council 1911–22, Honorary Secretary 1917–21, Vice-President 1923–5, Honorary Treasurer 1925–35, and, as a fitting conclusion, President 1935–7 He became a Trustee in 1938 and remained so until his death

Bagshawe was created C M G in 1915, and was knighted in 1933, he retired from the Bureau of Hygiene and Tropical Diseases in 1935

BOOK REVIEW.

"HAPPY TOIL"

By Major-General Sir LEONARD ROGERS, K C S I, KT, C I E, LL D, M D, F R C P, F R C S, F R S, I M S (retd)

Frederick Muller, London, 18*s* *Pages xvi* + 266

This fascinating volume tells the story of a long life, busy and filled with achievement The author's gift for original investigation became evident in the early days of his professional studies During his course of postmortem work as a third-year student, he met with three cases of multiple abscesses of the liver, due to suppuration of the bile ducts In one of these cases diagnosed as infected hydatids by the leading physician of the day, a long dissection enabled the student to trace the trouble to suppuration around an infected gallstone—" I never dared to tell the great man of my conclusions " Later, in India, he diagnosed the only case of this condition till then recognized there, and removed the offending gallstones, but, unfortunately, infection had already spread to the lungs (Moynihan later pointed out that Rogers was the first to discover and operate on this rare condition) His enthusiasm for research thus encouraged never flagged, in spite of the steep and thorny way that then confronted the pioneer He demonstrated the frequency of amoebic dysentery in India, and showed that tropical liver abscess is caused by amoebae,

a discovery that led to a revolution in treatment. His successful cultivation of the organism of kala azar is well known, and in 1913 after 20 years of labour he satisfied himself of the curative effect of antimony in this disease. In September 1914 everything was ready for an extensive trial of the drug but widespread riots necessitated the discharge of all his patients from hospital so that the work was not completed until May 1915 Unknown to Rogers at that time, two Italian doctors had reported 3 months earlier their successful treatment of infantile kala-azar in Sicily thus pre-dating his own announcement. And so the long story of research goes on—cholera, snake venoms studies on meteorological data in forecasting certain epidemic diseases, leprosy this last probably his most outstanding work for in Megaw's phrase, he is the man who brought hope to the leper"

In 1910 Rogers began his long crusade to establish a school of tropical medicine in Calcutta, a project that seemed to others in those days no more than a vision or a waking dream, and perhaps no one else in the face of such multitudinous and unconquerable obstacles, as they seemed, would have persisted. But courage and determination won through in the end, and when he finally quitted India in 1920 there stood in Calcutta a School of Tropical Medicine and Institute of Hygiene alongside an associated Hospital for Tropical Diseases, endowed with provision for "a staff of ten professors and seven whole-time research workers," a worthy memorial to a great man only to have been undertaken with a spirit that calls to mind the gallant challenge of the ruined but dauntless Scott—" Time and I against the world!"

One might go on quoting almost indefinitely from this enthralling narrative, but here no more can be said than to commend to others this life story of one of the company—as Virgil says—whose service to their kind has won them remembrance among men.

INDEX TO SUBJECTS

ANNOUNCEMENTS

NEXT MEETING OF THE SOCIETY

The 43rd Annual General Meeting of the Society will be held at Manson House at 7.30 p.m. on Thursday, 18th June, 1959.

The Manson Medal for 1959 will be presented.

An Ordinary Meeting will follow at 8 p.m. Dr FRANK HAWKING will read a paper entitled "Some recent work on filariasis".

An extra Meeting of the Society will be held at Manson House, 26, Portland Place, W.1, on Thursday, 4th June, 1959, at 7.30 p.m. Lieut.-Gen. Sir WILLIAM MACARTHUR will speak on "The story of smallpox in the British Isles".

MANSON LECTURE

To perpetuate the memory of the late Sir PATRICK MANSON, the Council of the Society has decided to establish a MANSON LECTURE FUND, to which subscriptions are now invited. It is hoped to raise a sum of at least £2,500, the accumulated interest from which will be devoted to financing a Manson Lecture.

The Lecture will deal with some aspect of tropical medicine or hygiene and will be given periodically by a recognized authority. The lecturer and the subject on which he will be invited to speak will be decided by the Council of the Society.

The Manson Lecture will be open to all members of the medical profession and will be advertised in the general medical press, in which it may be subsequently published.

MOVEMENTS OF FELLOWS

The following Fellows from abroad have notified the Secretaries that they are temporarily in the British Isles. Letters addressed to any of these care of the Royal Society of Tropical Medicine and Hygiene, Manson House, 26, Portland Place, London, W.1, can ... be forwarded to the home address.

To ensure the accuracy of this list, Fellows named below are particularly requested to advise the Secretaries when they return to their stations abroad.

[illegible]

[illegible]

[illegible]

[illegible], Nigeria

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible], Congo

[illegible] G., Trinidad

[illegible] Hon. Col. ... Malaya

[illegible] P. R., Nigeria

[illegible] D. I., Sudan

D... S. K., India

D... Lt.-Col. T. J., India

D... C. S., N. Rhodesia

D... F. G., Mauritius

GARROD, J. M. B., N. Rhodesia

GELFAND, M., S. Rhodesia

GOH, K. A., Hongkong

GOODCHILD, R. T. S., Uganda

GOSDEN, M., Sierra Leone

GREANY, W. H., Sudan

GREEN, R. T. B., Malaya

GUNTHER, C. L. M., New Guinea

HARGREAVES, F. H., Kenya

HENRY, J. A. F., Kenya

HENSHAW, L. P., Nigeria

HOWARD, A. C., Cyprus

HUNTER, W., Nigeria

JACKSON, Rosemary, Tanganyika

KELSEY, H. A., Nigeria

KENNEDY, Col. I. F., S. Rhodesia

KERTESZ, A., Nigeria

KHAN, P. N., India

Movements of Fellows—*Continued.*

Kuyper S W A., South Africa.
Lever R. J A. W., Malaya.
Low Nan-Wan Malaya.
Lwin R., Burma.
McKendrick, A. J Tanganyika.
Magid, M. A. Sudan.
Mok, Hing Yi Hongkong
Moos, B. S., India.
Nicholls, L., Singapore
Orenstein Maj.-Gen. A. J South Africa.
Pallister, R. A., Malaya.
Prasad Hari India.
Quintrell, D W Nigeria.
Richardson U F South Africa.
Ritchie, G L. Tanganyika.
Robinson, G G N Rhodesia.
Russell, A. F China.
Seevaratnam V J Malaya.
Sekar, S C., India.
Simpson T Nigeria.
Sur, M L., India.
To Shiu Yuen Hongkong
Upton B. H. B Fiji.
Van der Linde, P A. M., Hongkong
Watt G Gold Coast.
Wheaton, F L., Sudan.
Wilson Carmichael, Nigeria.
Wilson D Bagster, Tanganyika.
Zahir Albert British Cameroons.

NEW FELLOWS

At the meeting of the Society held at Manson House on 18th May 1950 the following 13 candidates were elected Fellows of the Society :—

A Deade, C. B., M.M.F (Bengal), C/o I C.L., India.
Banks, H. S Anley M D., F.R.C.P (Lond.), England.
Barwell, A. H., M.R.C.S. (Eng.), L.R.C.P (Lond.), Kenya.
Candler, P L M.B., CH. (Cantab.), Kenya.
Durran, Percy M.R.C.V.S. (Edin.) Sudan.
Gokberk, Celal, M D. (Istanbul), Turkey
Hopwood, G Maxwell, M.R.C.S. (Eng.) L.R .P (Lond.), England.
Humbert Walter C., M.D (Vanderbilt), M P.H. (Tulane) U.S.A.
Lovett W C. D M B., B CH. (Wales), M.D. (Lond.) D.P.H. (E) Somaliland
Montalvan, Juan A., M.D M.P.H., Professor Trop Med. University of Guayaquil
Noorooya, Hareen M M.R C.S. (Eng.) L.R.C.P (Lond), Mauritius
Pease, Clifford A., Medical Officer Inst of Inter American Affairs, Health and Sanitation Mission, Peru
Talbot Edmund J M D (New York), U.S.A.

ADMISSION TO FELLOWSHIP OF THE SOCIETY

All registered medical and veterinary practitioners and others interested in scientific pursuits relating to tropical medicine, whose qualifications are deemed satisfactory by the Council, are eligible for election as Fellows of the Society

Anyone desiring to become candidate for Fellowship of the Society should use the special form of application at the end of this journal.

The annual subscription payable by Fellows is one and half guineas (£1 11s. 6d.), which becomes due in advance on the 1st of April of each year

The Transactions and the current Year Book of the Society are posted regularly to every Fellow whose subscription is not in arrear

Further information may be obtained from the Hon. Secretaries, Manson House 26 Portland Place London, W 1 or from the Local Secretary of the district.

NOTICE TO FELLOWS

As a copy of each number of the TRANSACTIONS is posted to every Fellow whose subscription is not in arrear, Fellows are particularly requested to notify the Secretaries of any change in the address to which their TRANSACTIONS are to be posted

When copies of the TRANSACTIONS are returned by the Post Office marked "Gone Away," "No Service," or "Insufficient Address," no more copies will be posted to that address, but they will be retained at Manson House until further instructions are received

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LIBRARY NOTICES

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Happy Toil By LEONARD ROGERS London Frederick Muller, Ltd

Recent Advances in Chemotherapy By G M FINDLAY Vol I, 3rd Ed London J & A Churchill, Ltd

Schistosomiasis in South Central Africa By M GELFAND Cape Town Juta & Co, Ltd

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WAR-DAMAGED LIBRARIES, POST-WAR RESTORATION

Fellows will be rendering a service to the Society if they return to Manson House any numbers of the TRANSACTIONS which they do not wish to keep

The Council wishes to thank those Fellows who have already responded by returning copies of the TRANSACTIONS

PHOTOSTAT SERVICE

To help Fellows of the Society who cannot readily obtain access to medical periodicals and journals, arrangements have been made to supply "Photostat" copies of papers required

By courtesy of the Dean and Council of the London School of Hygiene and Tropical Medicine, the Library of the School has been placed at our disposal for this purpose Copies will be made by Photostat Limited We have also access to certain other libraries, but cannot guarantee to get copies of papers from the less known journals, though every effort will be made to do so

Fellows will be asked to pay only the actual cost of preparing the "Photostat" copy The minimum charge is 1s 3d per page copied (size $6\frac{1}{2} \times 11\frac{1}{2}$), but a price list will be sent on request

Application should be made to the Secretary, Royal Society of Tropical Medicine and Hygiene, Manson House, 26, Portland Place, W 1, giving the name of the journal, volume and page number of the article required Complete papers or specified portion of a paper can be supplied, but in the latter case lucid instructions must be given

Royal Society of Tropical Medicine and Hygiene.

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TELEPHONE LANGHAM 2127

ZONAE TORRIDAE TUTAMEN

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MANSON HOUSE,
26, PORTLAND PLACE,
LONDON, W 1

Number on Register of Fellows

APPLICATION FOR ADMISSION AS FELLOW OF THE SOCIETY

Name in Full (In Block Capitals)	Titles, Qualifications, etc , and their Source	Home Address *	Address Abroad *

* *When both addresses are given the address to which it is desired that communications be sent should be underlined*

PROPOSER — SECONDER

1 .... *Signature of Fellow* 2 .... *Signature of Fellow*

Extracts from Laws of the Society No 8 —"Either the proposer or the seconder must have personal knowledge of the candidate and vouch for him as in every respect suitable for election as a Fellow of the Society " No 24 —" Every Fellow shall pay an Annual Subscription of One-and-a-half Guineas (£1 11s. 6d) " No 25 —" The name of a newly elected Fellow shall not be placed on the Register of Fellows nor shall he be entitled to any of the privileges of Fellowship until after his first annual subscription [£1 11s. 6d] or composition fee [£23 12s. 6d] shall have been paid "

DECLARATION BY CANDIDATE.

I hereby undertake, if elected, to observe and obey the Laws and Regulations of the Royal Society of Tropical Medicine and Hygiene, and to endeavour to promote its honour and interests.

........................, 19 Signature of Candidate ....

FOR OFFICE USE

Application received ....

Approved by Council ....

Elected ....

First Subscription received ....

PUBLICATIONS OF THE SOCIETY

TRANSACTIONS

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Back Numbers Owing to the greatly increased demand for complete sets of the TRANSACTIONS, the stock of certain numbers had been exhausted These have now been reprinted, and any number can be supplied.

YEAR BOOK, 1950

List of Fellows (with Addresses) Alphabetically and Geographically arranged The Society's Annual Reports, Laws, and other matter

Price Five Shillings, post free

MONOGRAPH

Monograph I (September, 1936) "Boomerang Leg and Yaws in Australian Aborigines," by C J HACKETT 66 pages, 17 pages plates, stiff board cover, linen back Price 5s, post free

"MANSON CENTENARY"

Proceedings of meeting at Manson House on 12th December, 1944, with coloured portrait, price Two Shillings and Sixpence, post free.

"THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE"

An illustrated pamphlet by "Onlooker," describing the work and functions of the Society and the amenities of Manson House

Supplied free on application to the Secretary

Orders for the TRANSACTIONS and other publications of the Society should be sent direct to the Secretary, Royal Society of Tropical Medicine, Manson House, 26, Portland Place, London, W 1 *Telegrams* Anopheles, London *Telephone* Langham 2127 Cheques, Postal Orders, etc, should be made payable to the Royal Society of Tropical Medicine and Hygiene

The TRANSACTIONS may also be ordered through Messrs H K Lewis and Co Ltd, 136, Gower Street, London, W C 1, or any other bookseller

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ADVERTISEMENTS

Approved Announcements are accepted by the Council for insertion in the TRANSACTIONS, due to be published in alternate months as follows —

No 1	July 25th.	No 4	January 25th
No 2	September 25th	No 5	March 25th.
No 3	November 25th	No 6	May 25th

Advertisement type area 5 inches wide, 7½ inches deep (approx)

Half-tone illustrations—100 screen

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All communications regarding advertisements should be addressed to the Advertising Manager (S H Pilton)

EDITORIAL NOTICES

Papers submitted for publication in these TRANSACTIONS should be sent to the Hon. Secretaries Manson House 26, Portland Place London W.1.

The submission of matter for publication will be understood to imply that it is offered to this journal alone.

If accepted for publication, the copyright of papers becomes the property of the Society; but they may be re-published by permission of the Council, provided due acknowledgment be made of their having appeared in the TRANSACTIONS.

Papers should, if possible, be typewritten; they should be concisely written with subject matter logically arranged and sub-divided with references and abbreviations in the form described below; and with indications of the position, in the text, of illustrations, tables, maps, etc.

Titles should be as brief as consistent with clarity and in many cases the value of paper is enhanced by short summary at the end.

Temperature charts, graphs and drawings should be, if possible, in Indian ink on Bristol board; with detail and essential lettering large enough to be clearly legible after reduction if necessary (Write in pencil if lettering on drawing is to be set up and printed.)

Illustrations—if the number sent in is considered excessive, the author may be informed and given the opportunity of contributing to the cost.

Coloured plates are made only at the author's expense.

REFERENCES

In the text, the date of publication, in brackets, should follow the name of the author quoted thus:—

"T MANSON (1879) is due this epoch-making discovery

At the end of the paper list of References should be arranged in alphabetical order of authors surnames, and details given in the following order:—(1) Surname of author (2) Initials of author; (3) Year of publication in brackets, (4) Title of article, avoiding arbitrary capitals (The title of the article is sometimes omitted; but each list of references should in this respect be consistent throughout—giving all titles, or omitting all) (5) Title of journal; (6) Volume number; (7) Page number g:—MANSON, P (1879). On the development of *Filaria sanguinis hominis* and on the mosquito considered as nurse. *J Linn. Soc (Zool.)*, 14 304.

In the case of reference to book (1), (2) and (3) as above; (4) Title of book; (5) Edition and/or volume, if more than one; (6) Page number; (7) Town of publication; (8) Publisher's name, g:—*MANSON P (1898). Tropical Diseases*, 1st Ed 447 London Cassell & Co., Ltd.

Reference to an Annual Report SWAZILAND (1937). *Annual Medical & Sanitary Report 1936* p. 16.

Note The year of publication is not usually the year covered by the Report.

ABBREVIATIONS

The abbreviations used are those shown in the World List of Scientific Periodicals, 1934, which conforms to the rules of the *International Code of Abbreviations for Titles of Periodicals*, Paris, 1930. In general nouns have capital, adjectives small initial letters; articles, conjunctions and prepositions are omitted; the place of imprint is added only when uncertainty might arise, g

Amer J Hyg	*C R Acad Sci Paris*	*J Pharmacol.*
Ann. trop Med. Parasit	*C R Acad Sci Johannesburg*	*Ned. Tijdschr Geneesk.*
Arch Schiffs. Tropenhyg	*Dtsch med Wschr*	*Trans. R Soc trop Med. Hyg*
Bull Soc Path exot	*Indian med. Gaz*	*Z Hyg InfektKr*

The following contractions are to use whether the number to be expressed is 1 or more, g 1 c.c., 13μ, 42 kg):—

centigramme, cg.	kilometre km	millimetre, mm.
centimetre, cm.	micron, μ.	ounce, oz.
cubic centimetre	microgramme μg	pound, lb
cubic millimetre, mm.	milligramme mg	
kilogramme kg	millilitre, ml	

In order to avoid dangerous error in dosage gram and gramme are printed in full.

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